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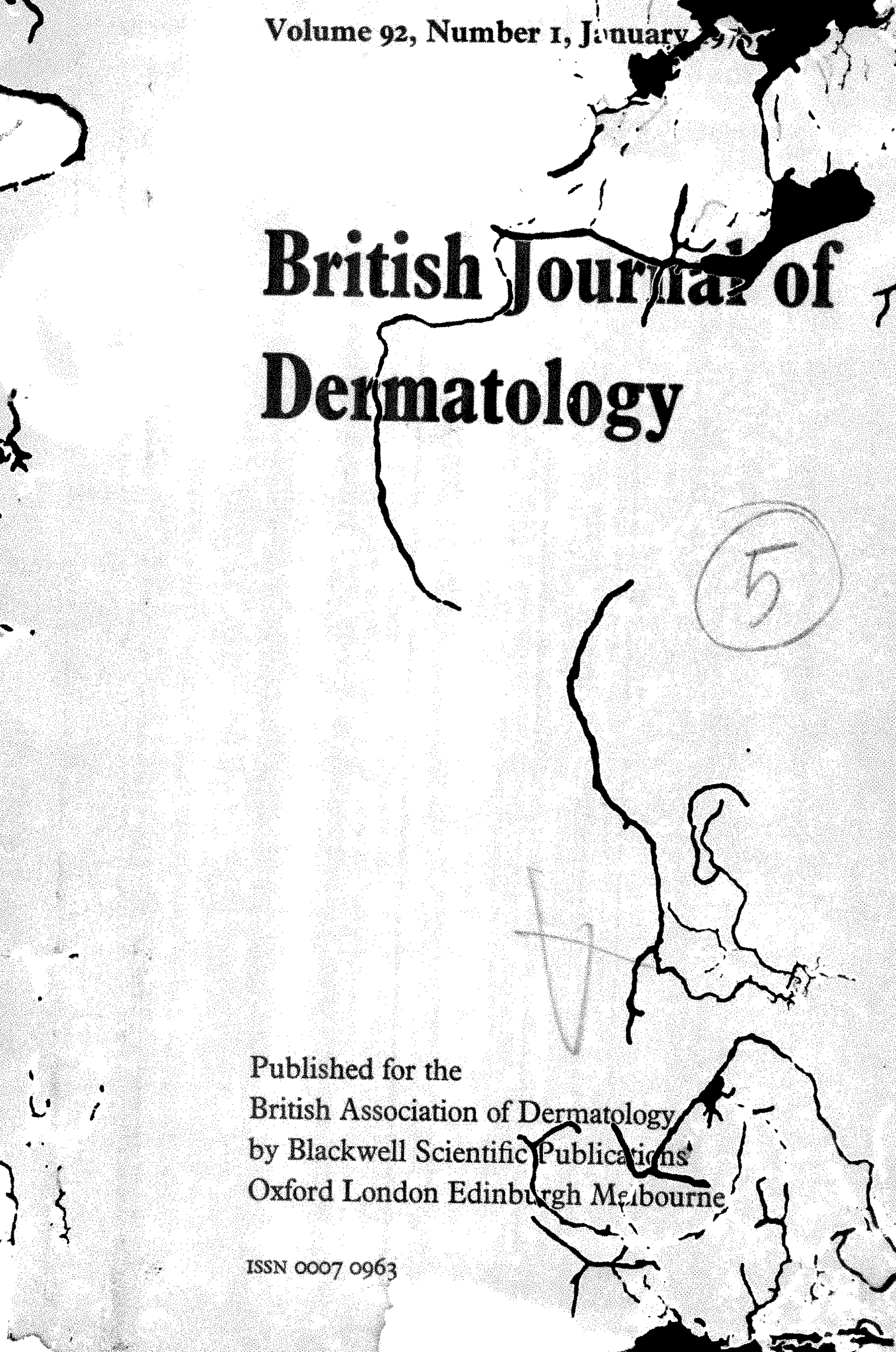
British Journal of Dermatology



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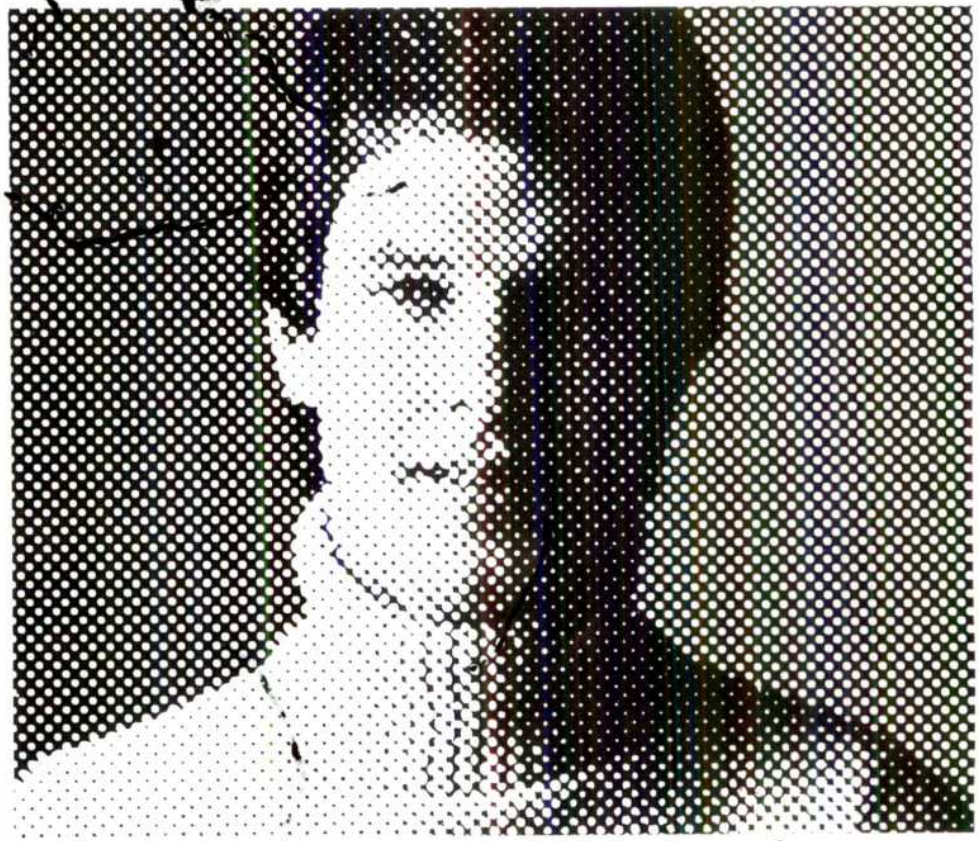

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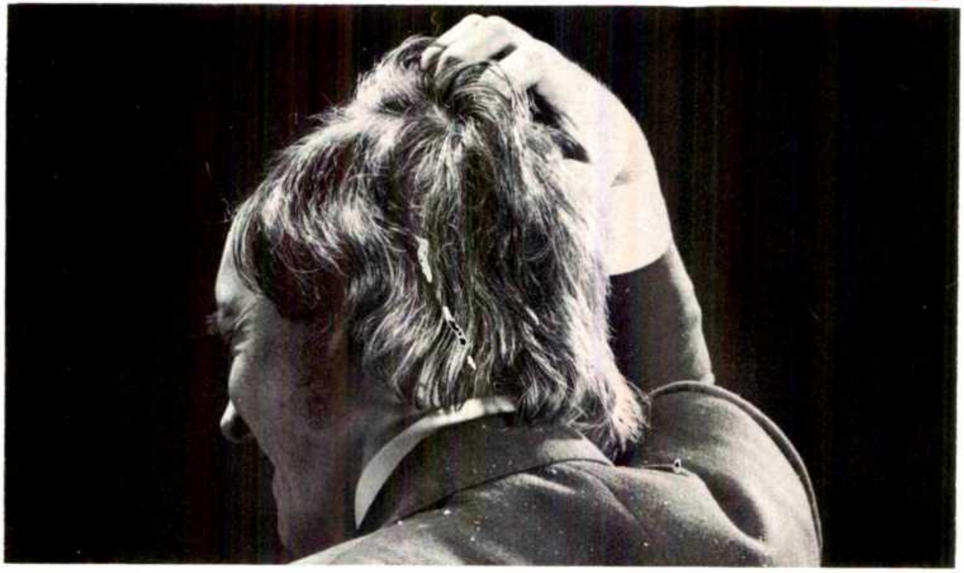

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Index Vol. 149, No. 1, 1974

Polano, M.K. (Leiden): Xanthomatosis and Hyperlipoproteinemia. A Review

Guerrier, C.J.W. and Wong, C.K. (Paris/Oxford): Ultrastructural Evolution of the Skin in Incontinentia pigmenti (Bloch-Sulzberger). Study of Six Cases

Weirich, E.G. und Longauer, J. (Basel): Topographische Unterschiede in der Ausbildung und Reaktivität der Aurikulartalgdrüsen des Kaninchens. II. Mitteilung. Histometrische Untersuchungen zur Talgdrüsenaktivität
(Topographical Differences in the Development and Reactivity of the Auricular Sebaceous Glands of the Rabbit. II. Communication: Histometric Studies of the Activity of the Sebaceous Glands)

Hägermark, Ö.; Wahlberg, J.E., and Germanis, M. (Stockholm): Determination of Oxyquinoline Concentrations in Plasma in a Patient Treated for Acrodermatitis enteropathica – an Aid in Therapeutic Control

Hellgren, L. and Vincent, J. (Göteborg): Skin Surface Retention of an Applied Ointment

Bhutani, L.K.; Bedi, T.R.; Pandhi, R.K., and Nayak, N.C. (New Delhi): Lichen planus pigmentosus

Clayton, R. and Wood, P. (London): Subcutaneous Nodular Sarcoid

The Interesting Case · Der interessante Fall · Un cas intéressant

Hopf, B. und Krebs, A. (Bern): 'Ulcera cruris' als seltene Form einer Sarkoidose
('Ulcera cruris' as a rare manifestation of sarcoidosis)

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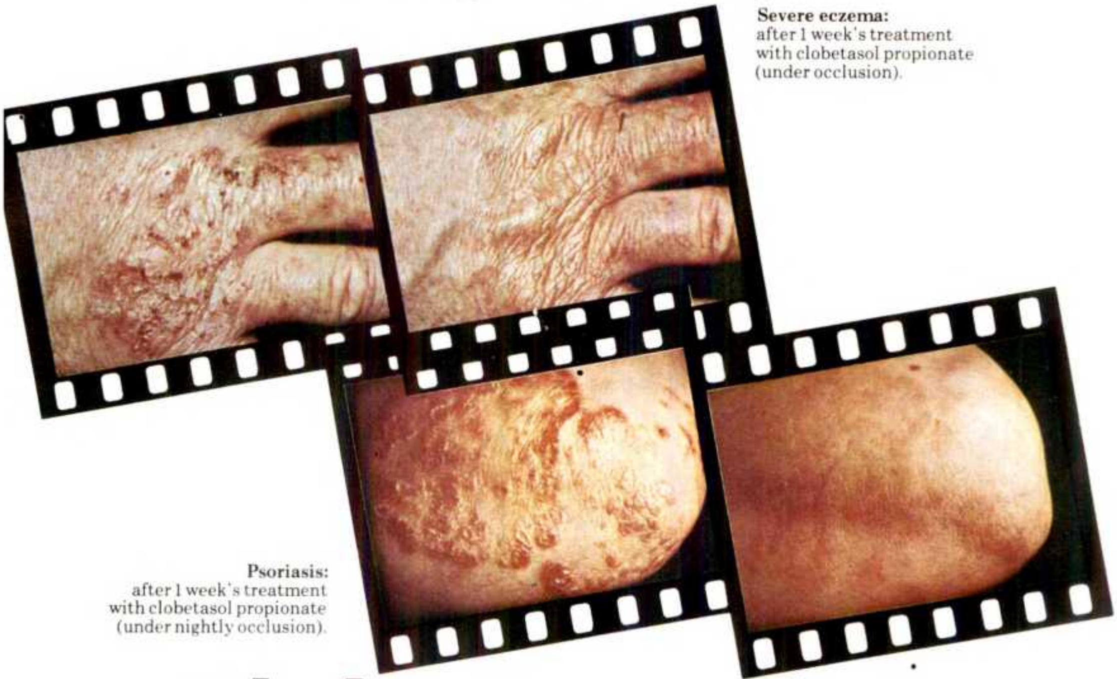

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Reference 1 Olansky, S.; in *Current Therapy;* edited by H F. Conn, London: Saunders, 1969, p.646.

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1. Almeyda, J. and Burt, B.W., *Brit. J. Derm.* (To be published) 2. Harries, E. H. L. and Jacoby, R. H. (To be published)

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Edited by

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F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

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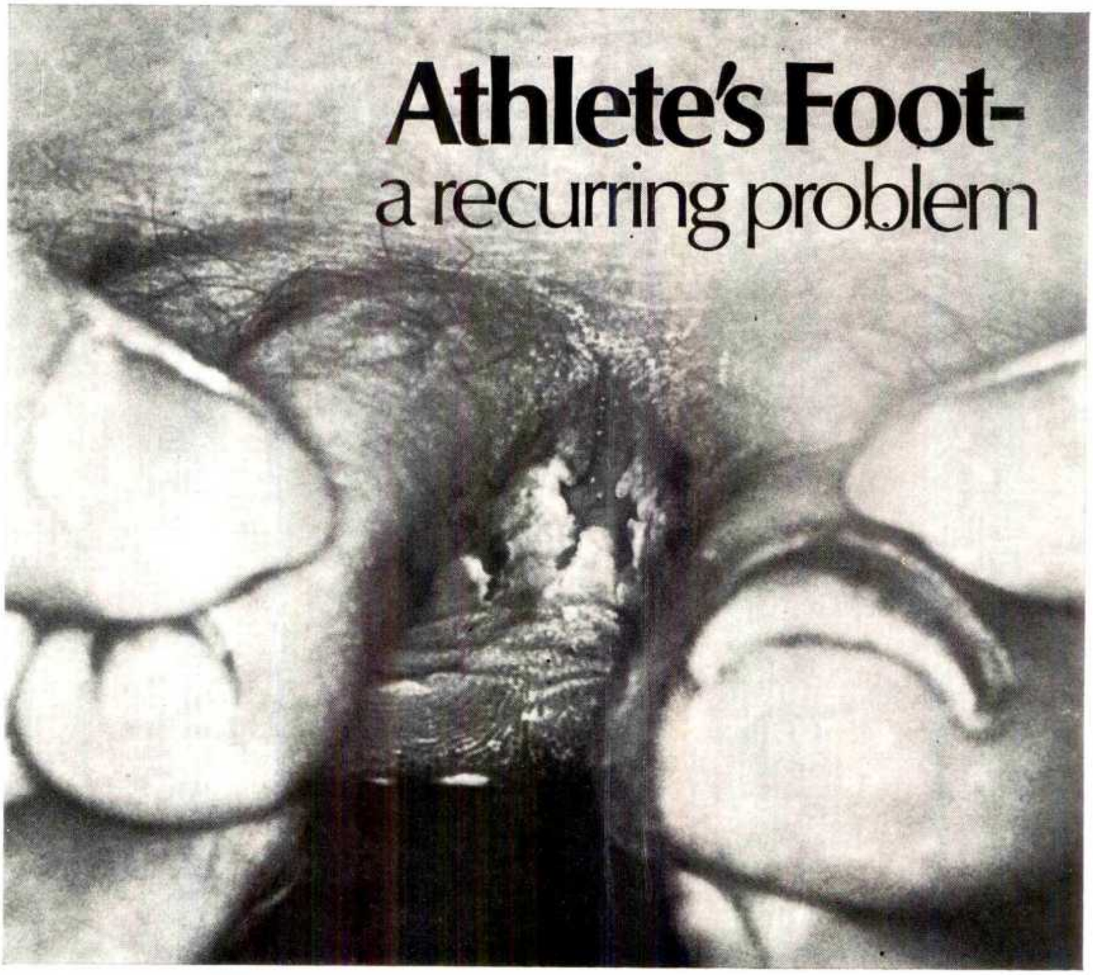

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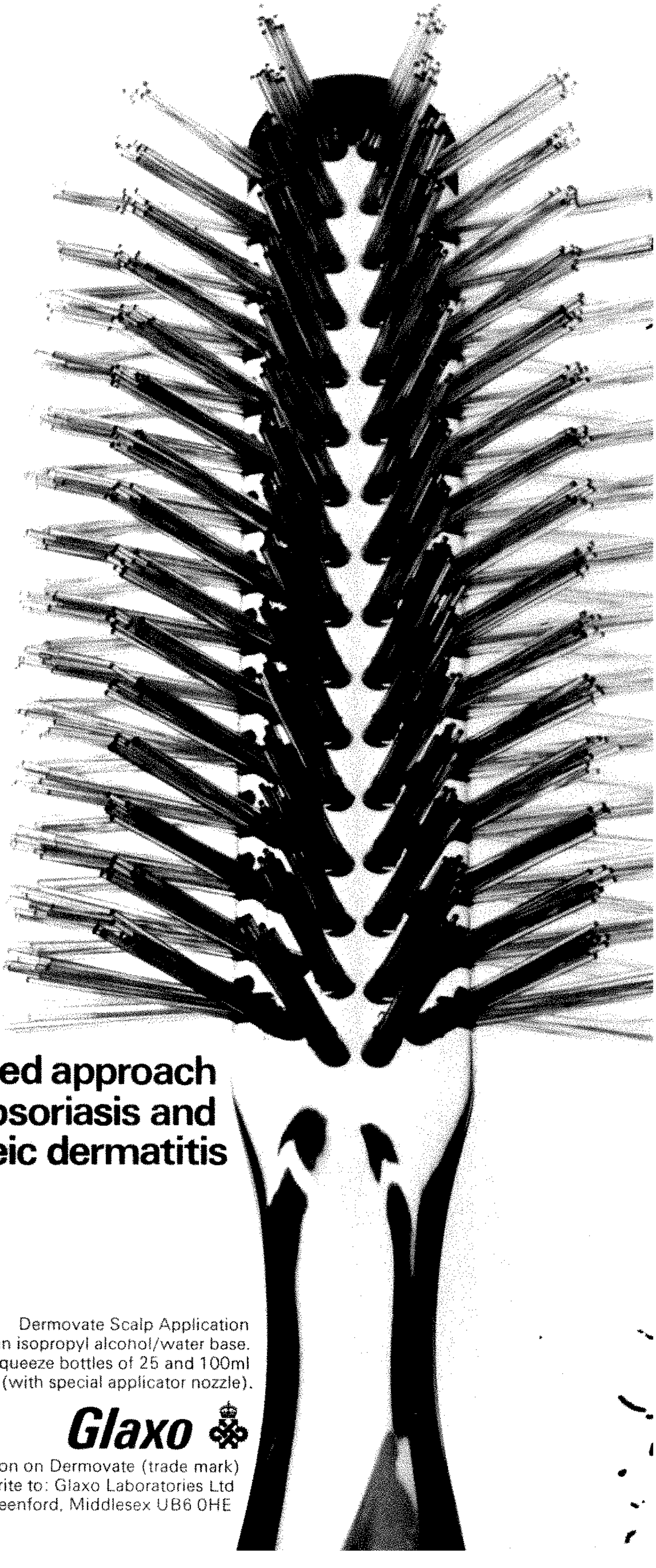

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British Journal of Dermatology (1975) **92**, 1.

Electrical potential difference between basal cell carcinoma, benign inflammatory lesions and normal tissue

R.E.WOODROUGH,* G.CANTI† AND B.W.WATSON*

Department of Medical Electronics*, Department of Histopathology,† St Bartholomew's Hospital,
West Smithfield, London EC1

Accepted for publication 24 July 1974

SUMMARY

Measurement of the resting electrical potential difference on the surface of the skin has been made on patients with suspected basal cell carcinoma. It was demonstrated that in fifty-five lesions from forty-nine patients, the mean electrical potential difference from thirty-six basal cell carcinomas was found to be +11 mV, from nineteen benign inflammatory lesions it was found to be +2 mV, and from normal skin, contra-lateral to the lesions in both groups, it was found to be −3 mV. All the measurements are relative to a reference electrode placed under the tongue. The difference between the means for the inflammatory lesions and for the normal skin was found to be statistically insignificant, but the difference between these means and the mean potential difference for the basal cell carcinoma group was found to be highly significant ($P<0{\cdot}1\%$). However, the populations overlap to such an extent as to make the measurement, in its present form, useless as a diagnostic technique.

It is a well established fact that potential differences can be found, varying in magnitude and sign, between any two points on the skin of the human body. Indeed, these potential differences also exist between various organs of the body, and the phenomenon is not restricted to man (Becker, Bachman & Friedman, 1962; Schauble & Habal, 1969). The magnitude of the potential difference (p.d.), which varies from zero to about 100 mV, may be divided into two components. A relatively fast varying component which results from an applied stimulus, and a component which represents the resting level. Psychologists make use of the first component in assessing emotional responses. Known as the skin potential response, this component is thought to be primarily due to eccrine sweat gland activity. The second component, which is the component dealt with in this report, is of unknown origin; although a number of hypotheses have been presented. The resting potential difference represents the algebraic sum of a number of competing factors, but we believe the predominant factor to be a measurable spatial variation in the ionic composition of the plasma. In addition to this, various semi-permeable membranes throughout the body might be expected to add a significant contribution, and there is some evidence that the skin acts as a semi-permeable membrane.

Observations by Melczer & Kiss (1957) and the work of investigators such as Langman & Burr (1949), led them to investigate the potential difference associated with epithelial neoplasms. They found basal cell carcinomas to have a positive electrical potential relative to normal tissue, which appears to conflict with other reports. Langman & Burr (1949) recorded cervical cancers to be negative with respect to the ventral abdominal wall, and in the hamster Schauble & Habal (1969) found induced tumours to be consistently negative compared to various internal organs. Dalgeard & Thygesen (1950) were unable to confirm Langman & Burr's findings. In only 30% of thirty-three proven cases of uterine carcinoma did they find the cervix to be negative with respect to a reference electrode placed on the skin slightly above the symphysis pubis. Unfortunately, they did not publish any figures for a control group. Langman & Burr (1949) did discuss results for a control group, however they did not present any figures for either the malignant or the non-malignant group.

A careful study by Burrows, Roe & Schober (1945) using induced skin tumours in mice showed a statistically significant difference between the mean p.d.'s for experimental animals arranged in groups according to the histological stage reached at death. These p.d.'s also differed significantly from a control group. It was specifically stated that according to their study the technique was of no use in clinical diagnosis, since they could not prove significant differences in individual animals.

This report describes results from part of a study undertaken to resolve the nature of the skin electrical potential difference and the changes in p.d. associated with neoplasia. Suspected basal cell carcinomas were chosen for our experiments because they were easily accessible, and allowed us to compare our results with those of Melczer & Kiss (1957). Special attention was paid to the elimination of measurement artifacts, and to the validity of the cytological evidence which was re-examined after the successful treatment of the lesions.

MATERIALS AND METHODS

Description of electrodes

Plastic 5 ml syringes were adapted to make electrode holders. Fig. 1 illustrates the way in which an electrode can be mounted in a syringe; the soldered connection to the electrode is kept out of contact with the electrolyte by means of the rubber plunger.

Electrodes for mounting on the end of the plunger were made from 20 S.W.G. silver wire, bent to shape and then chlorided in a manner similar to that used by Coles & Binnie (1968); cleanliness is essential and a very low chloriding current is required to ensure a stable chloride layer and a low residual bias potential (~0·1 mV) between electrode pairs. The electrode is easily pushed through the rubber plunger to form the completed assembly.

The probe electrode used an electrolyte bridge formed from physiological saline (154 mM NaCl) and sodium methylcellulose. Sodium methylcellulose, which may be sterilized by heating the powder in an autoclave, can be dissolved in cold saline and was used to increase the viscosity of the solution, enabling measurements on the skin to be positioned accurately. The mouth reference electrode used physiological saline (154 mM NaCl) for the bridging electrolyte. A small length of non-toxic vinyl tubing plugged at one end with cotton wool was attached to the end of a syringe electrode holder. The cotton wool plug was moistened by pushing the syringe plunger, and then placed in position under the tongue.

After use, the vinyl tubing was removed from the reference syringe electrode holder and disposed of. Cross-contamination of the probe syringe electrode holder was similarly avoided by using a shorter length of vinyl tubing. No cotton wool plug was necessary for use with the probe electrode.

It was important when making measurements to ensure that the bridging electrolyte was sufficiently

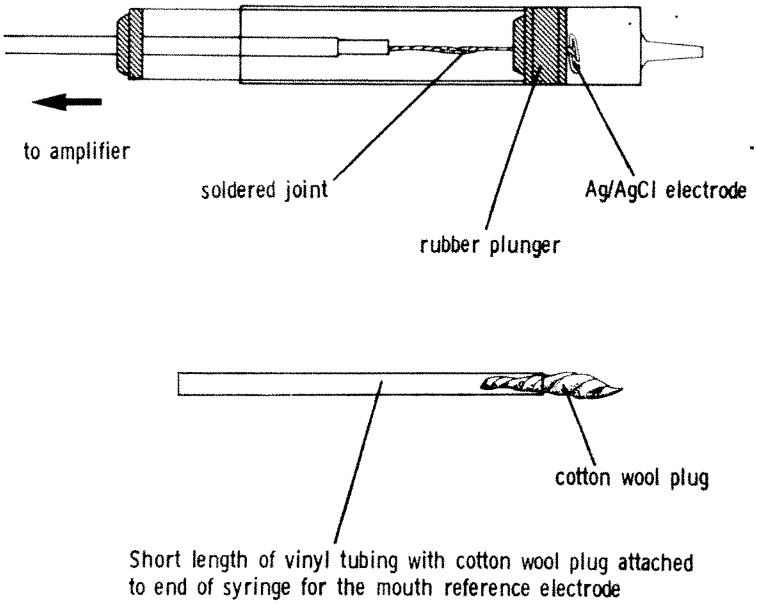
to amplifier
soldered joint
rubber plunger
Ag/AgCl electrode
cotton wool plug
Short length of vinyl tubing with cotton wool plug attached
to end of syringe for the mouth reference electrode

FIGURE 1. Diagram of the electrodes.

free of air-bubbles. The condition of the electrolyte was easily observed through the clear plastic of the syringe. A variation in potential difference has been observed which arises if the probe is pressed onto the surface of the skin; to avoid this artifact it was necessary to squeeze out the electrolyte gel to contact the skin, maintaining a small gap between the end of the probe and the skin. A simple analysis, described below, indicates that the concentration of the bridging electrolyte should not alter the result providing that the electrolyte composition is not varied far from the normal physiological range. Filling the syringes with electrolyte was, of course, achieved by operating the syringe in the usual manner.

Measurements

Potential difference measurements were made using a high input impedance voltmeter.* The probe electrode was placed in contact with the skin at the site of interest, and the voltage with respect to a mouth reference electrode was recorded. The p.d. of a pathological site was compared with a contralateral 'normal' site. There was no preparatory cleansing of the skin, the assumption being that the normal site would act as a control. Measurements were made from a total of seventy-three patients on 100 suspected basal cell carcinomas presented for cytological examination over an 18 month period. The nature of the lesions was not known to the physicist making the potential measurements.

The technique adopted was to make the measurement with the patient seated. Patients were set at ease by explaining the procedure. The tube attached to the reference electrode was then placed under the tongue and held in position by the patient. It was not necessary to remove dentures and the position of the tube was not critical. In order to avoid any risk of contamination the normal reference site was always measured first and then the lesion. The validity of a reading could be checked by removing the probe and then placing it back in contact with the skin.

Fig. 2 illustrates the readings which were taken and their relationships. Notice that, provided the same electrode holder is used to contact the skin at the different sites, the electrolyte does not evoke a physiological response, and that as attention is confined to the value of $(X-Y)$, it should not matter what the concentration of the electrolyte is. If the half-cell potential between the electrolyte and the dermis at a given site is assumed to be of the form $A \ln \left(\frac{C_e}{C_i}\right)$, where C_e refers to the concentration of transferable ions in the electrolyte bridge and C_i the concentration of that species of ions in the dermis, and A is a constant, then the potential difference $(X-Y)$mV between any two sites is always of the form $A \ln \left(\frac{C_i(1)}{C_i(2)}\right)$, where $C_i(1)$ and $C_i(2)$ refer to the concentrations at the two sites. It is worth stressing that this technique also serves to eliminate the artifact caused by any residual bias potential which may exist between the electrodes.

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*Levell d.c. multimeter, type TM9B.

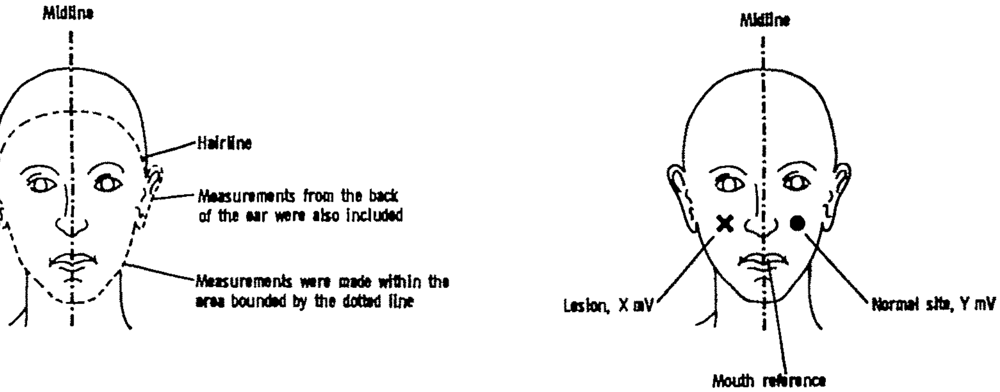
Midline
Hairline
Measurements from the back of the ear were also included
Measurements were made within the area bounded by the dotted line
Midline
Lesion, X mV
Normal site, Y mV
Mouth reference

FIGURE 2. The area used in the study, and the relationship between the readings. Only lesions within the area bounded by the dotted line, and having a contra-lateral normal site were included in the analysis. Mouth reference to lesion, X mV; mouth reference to normal site, Y mV; lesion to normal site, (X−Y) m V.

Care was taken that the lesions used in the statistical analysis were either basal cell carcinomas or could be described as benign inflammatory lesions, and only lesions falling within the area shown in Fig. 2 were considered. Of these only lesions having a contra-lateral normal site were included, excluding fifteen lesions which fell on the mid-line or formed contra-lateral pairs of lesions. As an additional precaution, no lesion noted as being badly ulcerated was used. The precaution of limiting the measurements used in the analysis to the facial area was taken because of the assumption of a constant relationship in p.d. between contra-lateral sites. We considered that this simple assumption was unlikely to be valid if other areas of the body were included. Results from two patients were discarded because it was noted that the readings were unsteady; it is possible that this was caused by air bubbles in the tube attached to the reference electrode, or by the patients changing the position of the reference electrode in their mouth. Movement of the reference electrode was not normally observed to be a problem during the short time it took to make a measurement.

RESULTS

Measurements on fifty-five lesions complying with the criteria outlined in the method were analyzed to determine whether basal cell carcinomas showed a significantly different p.d. from normal tissue

TABLE 1. Summary of results

Inflammatory lesion group $n = 19$	Mean (mV)	Standard deviation (mV)
Normal site–mouth (Y)	− 3·3	19·9
Lesion–mouth (X)	+ 1·6	12·6
Lesion–normal site (X − Y)	+ 4·9	16·4
Basal cell carcinoma group $n = 36$		
Normal site–mouth (Y)	− 3·6	19·1
Lesion–mouth (X)	+ 10·8	14·8
Lesion–normal site (X − Y)	+ 14·4	13·0

or benign inflammatory lesions; insufficient squamous cell carcinomas were seen during our study to allow comment on any possible differences between basal cell carcinoma and squamous cell carcinoma. The physicist responsible for making the potential measurements submitted the names of the patients to be used in the analysis to the cytologist, who grouped the lesions into thirty-six basal cell carcinomas and nineteen benign inflammatory lesions. The statistical analysis was then completed by the physicist. At no time did the cytologist have access to the potential difference measurements or the physicist to the cytological information. Student's t-test was applied to test for a significant difference between the means, a matched-pairs test being applied to the values of (X − Y), and a 2-sample t-test being applied to test for significance in the other results. The validity of applying the t-test was verified by performing an F-test on the variances.

The results of the statistical analysis are outlined in Table 1. The sample mean (X − Y) for the basal

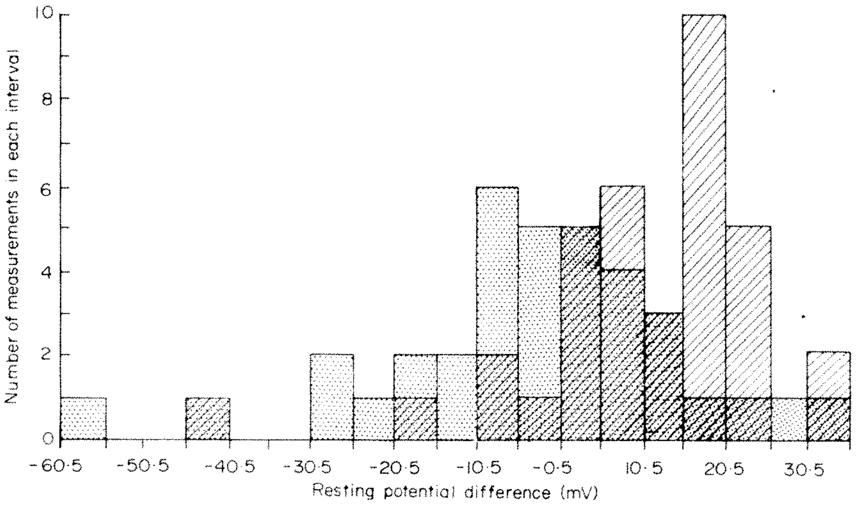

FIGURE 3. Histograms of XmV and YmV for the basal cell carcinoma group.
XmV hatched; YmV stippled.
Hatching and stippling together has no special significance and is due to overlap of the two histograms.

cell carcinoma group was found to be highly significant ($P<0{\cdot}1\%$), whereas the sample mean (X – Y) for the benign inflamatory lesions group was found to be non-significant ($P>20\%$). It was also confirmed that there was no significant difference between the mean p.d. for the contralateral normal sites of the two groups ($P>50\%$). Histograms of the electrical potential difference associated with basal cell carcinomas and contra-lateral normal sites are presented in Fig. 3. The mouth was used as a zero reference point. It was expected that measurements of X and Y would be subject to a large variance because of the lack of subject to subject control, an artifact due to the residual bias potential between the probe and reference electrodes, and because of differences in the reference voltage associated with different positions in the mouth. Despite these objections, the data have been presented because they do provide evidence of a difference in p.d. between normal and neoplastic tissue. Furthermore, a comparison of the mean values of X for the basal cell carcinoma group and the benign inflammatory lesions group showed a significant difference with $P<0{\cdot}1\%$.

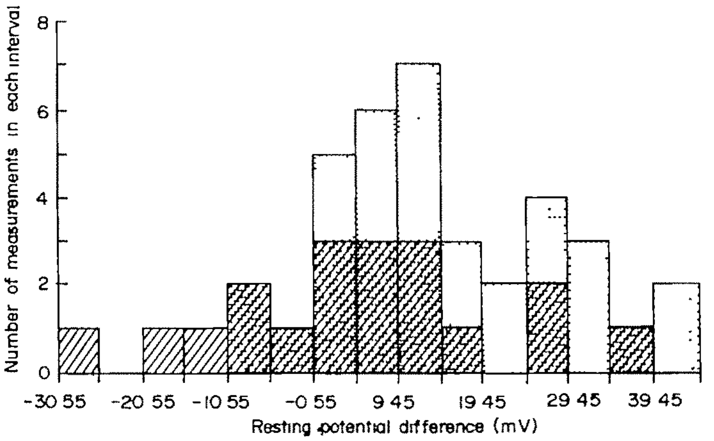

FIGURE 4. Histograms of (X – Y)mV for the basal cell carcinoma group stippled and the benign inflammatory lesions group hatched. Hatching and stippling together has no special significance and is due to overlap of the two histograms.

We attempted to avoid the problems associated with the data presented in Fig. 3, by comparing the electrical p.d. between the lesion and a contra-lateral site (X – Y)mV for the basal cell carcinoma group, and the inflammatory lesion group. It was the possibility of distinguishing between these groups that was of prime clinical interest. Histograms of (X – Y) are presented in Fig. 4. Again, a highly significant difference is found between the means of the two histograms ($P<0{\cdot}1\%$), but the variances are disappointingly large. The large variance on the measurement of (X – Y) for the basal cell carcinoma group could indicate a failure in the method of subject to subject control, due either to the assumption of a constant relationship in p.d. between contra-lateral sites, or to the assumption of negligible variation with time.

DISCUSSION

As shown in Table 1, the mean electrical p.d. associated with basal cell carcinoma is found to be 9 mV more positive than the mean p.d. associated with benign inflammatory lesions, and 14 mV more positive than the mean p.d. of normal tissue, in general agreement with the result of Melczer & Kiss (1957). However, although we consider our results to be of considerable academic interest, like

Burrows, Roe & Schober (1945), we find the populations overlap with large variances, and cannot agree with Melczer & Kiss (1957) that this technique, in its present form, is reliable for diagnosing basal cell carcinomas. The discrepancy between these results and those of Schauble & Habal (1969), Langmann & Burr (1949) and Burrows *et al.*, (1945), over the sign of the potential difference, is difficult to explain. It is possible that the result is peculiar to epithelial neoplasms such as basal cell carcinomas. We have not investigated other neoplasms and, although Melczer & Kiss (1957) made measurements on seven cervical carcinomas, such a small number does not have any significance. Melczer & Kiss (1957) used KCl as a bridging electrolyte but we used NaCl in common with the other investigators, eliminating the possibility of the difference being due to the electrolyte used. It is well known that injury to the dermis leads to an increase in potential difference, the injured area being positive with respect to the uninjured site. This phenomenon would further complicate diagnosis, but it is unlikely to be responsible for the result of our work in which we compared basal cell carcinomas with other inflammatory lesions.

The stability of the readings has been checked over time intervals of up to 1 h but long term variation has not been fully examined. Measurements were made on patients at random times during the working day, and this could be one reason for the large variances. The large variances must also reflect the complexity of the components of the resting electrical potential measured on the body's surface, but there is also no doubt that making spot measurements and assuming a symmetrical relationship between contra-lateral sites aggravated the situation. Potential contour maps would probably increase the precision of a diagnosis, but using the present technique such maps would be tedious and time consuming to produce.

It is intended to extend this study to other neoplasms, and in particular to investigate the possibility of using this measurement to monitor therapy.

ACKNOWLEDGMENTS

We wish to express our thanks to Dr D.D.Munro and Dr P.F.Borrie for allowing their patients to be used in this study, to Mr D.Smith for advice on the statistical analysis, and to staff of the departments of Histopathology and Medical Electronics at Saint Bartholomew's Hospital for providing us with valuable assistance. We are also pleased to acknowledge the co-operation given by the many patients who have made this report possible, and the Department of Medical Illustration at Saint Bartholomew's Hospital for producing the artwork.

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Lymphocyte transformation test in allergic contact nickel dermatitis

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SUMMARY

Twenty-five patients with clinically proven, patch test positive nickel dermatitis were studied with the lymphocyte transformation test (LTT). Serial dilutions of $NiSO_4$ were present in the lymphocyte culture. None of the controls showed lymphocyte blast transformation. In fourteen patients maximal incorporation of thymidine occurred at the highest dilutions (1:50,000–1:100,000). In eleven patients the highest incorporation occurred at the lower dilutions. The nature of the antigen responsible for the specific reaction remains unknown.

Allergic contact dermatitis due to nickel hypersensitivity has commonly been studied by the patch test method using an aqueous solution of nickel sulphate. Several workers have used the lymphocyte transformation test (LTT) in patch test positive nickel sensitive subjects (Aspegren & Rorsman, 1962; Pappas, Orfanos & Bertram, 1970; MacLeod, Hutchinson & Raffle, 1970· Hutchinson, Raffle & MacLeod, 1972; Forman & Alexander, 1972), seeking a more sensitive technique for the detection of contact hypersensitivity.

In an attempt to find a better understanding of some of the reported results, we have investigated the effect of using various doses of nickel sulphate in the LTT. This nickel salt is one for which there are no published reports of non-specific stimulation of lymphocytes.

MATERIALS AND METHODS

Twenty-five patients with clinically confirmed nickel contact dermatitis were studied. All patients were patch tested on the skin of the upper back with a 2·5% (0·16 mol/l) aqueous solution of nickel sulphate ($NiSO_4$, $7H_2O$), (Merck), 0·25 ml (0·3 mmol), spread on an area of 1 cm^2 and read after 48 h. On removal of the patch all patients showed a vesicular reaction which persisted for several days. LTT was performed on each patient using serial dilutions of nickel sulphate. Ten normal people without a history of eczema and with a negative patch test to $NiSO_4$ were used as controls.

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Lymphocyte Transformation Test Technique

Twenty ml of human defibrinated blood were mixed with an equal volume of a buffered (pH 7·2) isotonic saline solution. Six ml of the diluted blood were poured into sterile tubes 15 × 100 mm with screw caps (Sovirel, France) containing 3 ml of a mixture of Pielograf-Ficoll. After 20 min of centrifugation at 1800 rpm in a horizontal centrifuge (MSE Minor) at room temperature the lymphocytes appeared at the plasma Pielograf-Ficoll interphase. The cells were aspirated with a Pasteur pipette and 95% found to be lymphocytes. These cells were next washed three times with the culture media (see below) and adjusted to 5×10^6 per ml.

Pielograf (Juste, Madrid) of a concentration of 76% was added to an 8% aqueous solution of Ficoll (Pharmacia, Upsala) to achieve a density of 1080. The mixture was autoclaved in a 600 ml vacuum bottle (Baxter).

Culture medium was made up by mixing the following ingredients:

(1) 50 ml of 'Medium 199—with Earle's Salts 10' (Flow).
(2) 12 ml of hepes (Sigma) 1M in distilled water to which NaOH 1M was added to achieve a pH of 8.
(3) 450 ml of distilled water.
(4) 50 ml of heat-inactivated fetal calf serum (Gibco).

A 1% solution of $NiSO_4, 7H_2O$ (Merck) in saline (pH 7·2) was sterilized by filtration and stored at 4°C until used. From this stock solution serial dilutions of 1:1000 ($0{\cdot}65 \times 10^{-2}$ mol/l), 1:5000 ($1{\cdot}3 \times 10^{-3}$ mol/l), 1:10,000 ($0{\cdot}65 \times 10^{-3}$ mol/l), 1:50,000 ($1{\cdot}3 \times 10^{-4}$ mol/l), 1:100,000 ($0{\cdot}65 \times 10^{-4}$ mol/l) were obtained using as diluent the cell culture medium. When performing the LTT the serial dilution tubes were set up in duplicate and 4 ml of the appropriate strength added to each.

The cell suspension was taken and 0·1 ml added to screw-capped tubes which contained 4 ml of either (a) the $NiSO_4$ serial dilutions, (b) culture medium only, (c) culture medium with 0·1 ml of phytohaemagglutinin-P (Difco) 1:10. All tests were carried out in duplicate. The tubes were kept at 37°C for 96 h at which time 2 μCi of tritiated thymidine were added (specific activity 20·5 Ci/mmol, The Radiochemical Centre, Amersham, England). Incubation was allowed to proceed for another 16–18 h and the contents were then centrifuged for 10 min at 3000 rpm. The supernatant was discarded and the cellular sediment resuspended in cold saline. This suspension was then passed through a 25 mm glass filter (25 mm Sartorius), using a water suction pump containing Whatman filters typeGF/C 2·4 cm in diameter.

The cellular deposit in the filter was then treated successively with 20 ml of saline, 40 ml of 5% cold TCA and 20 ml of 50% methanol. The filters were placed in a vial of a liquid scintillator and dried at 120°C for 60 min. Next, 10 ml of scintillation fluid (0·0379 g of POPOP, 22·74 g of PPO and 3·79 l of toluene) were added to each vial, which were then placed in a liquid scintillation spectrophotometer for counting (Nuclear Chicago, model Mark II).

RESULTS

In ten normal individuals the response of the lymphocytes to PHA was within normal limits (Table 1). When serial dilutions of $NiSO_4$ were present in the lymphocyte culture the average response obtained was consistently below the spontaneous thymidine uptake in the control tubes. Results on a graph appear in Fig. 1.

The response of the patients' lymphocytes to PHA was within normal limits. Two types of specific response were observed in the group of twenty-five patients.

In fourteen patients, when serial dilutions of $NiSO_4$ were present in the lymphocyte culture, the maximal incorporation of thymidine occurred at the highest dilutions (Table 2). At the higher concentrations (1:1000–1:5000) the cpm were equal to or below the ones obtained in the control tubes. At a dilution of 1:10,000 the cpm started showing a rise, attaining a maximum in either the 1:50,000 or the 1:100,000 dilution. Patient III showed 89 cpm at a dilution of 1:1000; 170 cpm at 1:5000; 3,383 cpm at 1:10,000; 6,099 cpm at 1:50,000; peaking at 54,835 cpm at 1:100,000. In several patients the lower dilutions gave a cpm below the corresponding control tube, lymphocyte blast transformation occurring only in the higher dilutions. This group of patients could be considered 'high dilution responders'. A graph of the results obtained appears in Fig. 2.

TABLE 1. LTT in ten normal individuals with serial dilutions of $NiSO_4$ in the lymphocyte culture

Cases	Counts per minute						
	Tube control	Lymphs. P.H.A.	$NiSO_4$ Dilutions				
			1:1000	1:5000	1:10,000	1:50,000	1:100,000
I	1861	43,299	1527	2118	1175	—	—
II	730	125,529	82	114	860	760	680
III	1229	127,363	63	76	131	47	48
IV	1692	166,523	132	119	262	202	1832
V	473	54,153	55	113	111	181	173
VI	1045	60,322	61	47	27	41	48
VII	691	34,732	75	92	418	258	112
VIII	1334	64,389	80	88	172	192	166
IX	739	199,999	127	105	127	163	—
X	701	100,763	345	382	417	644	555
Mean	1049	97,707	255	325	370	276	452
Standard deviation	148	17,699	144	201	117	84	214

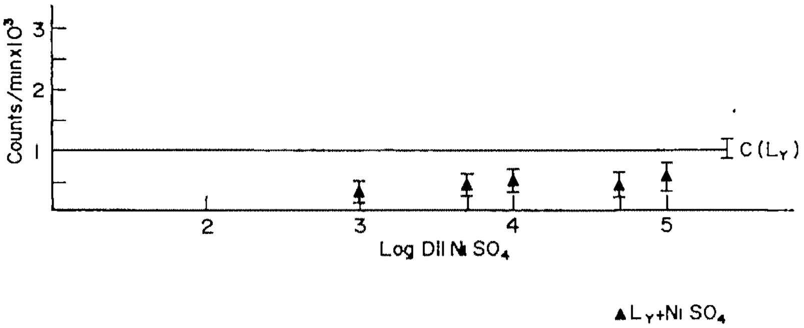

FIGURE 1. Mean and standard deviations of thymidine incorporation in the LTT in ten normal controls with serial dilutions of $NiSO_4$ in the lymphocyte culture.

Conversely, in eleven patients the highest uptake of thymidine was noted at the lower dilution, while at the higher ones the incorporation was less active or completely absent (Table 3). Patient III showed 6,693 cpm at a dilution of 1:1000, 4,763 cpm at 1:5000, 3,629 cpm at 1:10,000, 1,112 cpm at 1:50,000,

TABLE 2. LTT in fourteen nickel sensitive patients with serial dilutions of $NiSO_4$ in the lymphocyte culture. 'High dilution responders', the maximal incorporation of thymidine occurring at the highest dilutions of $NiSO_4$

Cases	Counts per minute						
	Tube control	Lymphs P.H.A.	$NiSO_4$ Dilutions				
			1:1000	1:5000	1:10,000	1:50,000	1:100,000
I	2601	119,575	2256	2920	3533	3534	3210
II	2500	138,880	1104	1185	1680	3303	11,313
III	1784	110,763	89	170	3383	5099	54,835
IV	3670	15,242	—	39	202	2411	15,303
V	1996	60,518	165	332	4468	3865	2686
VI	1298	21,772	288	259	924	3277	2653
VII	315	71,329	527	691	40,983	42,320	41,312
VIII	3555	46,700	75	275	2505	4185	5905
IX	978	85,614	72	688	1293	3251	3694
X	1724	64,316	201	1116	9882	12,721	17,244
XI	1174	72,111	227	648	8743	10,562	15,428
XII	1920	83,247	532	1100	9872	9997	13,220
XIII	2410	54,820	—	1328	5420	30,240	15,220
XIV	1268	60,340	112	296	1130	42,320	10,340
Mean	1949	71,802	470	789	6715	12,649	15,168
Standard deviation	253	9245	183	198	2769	3879	4044

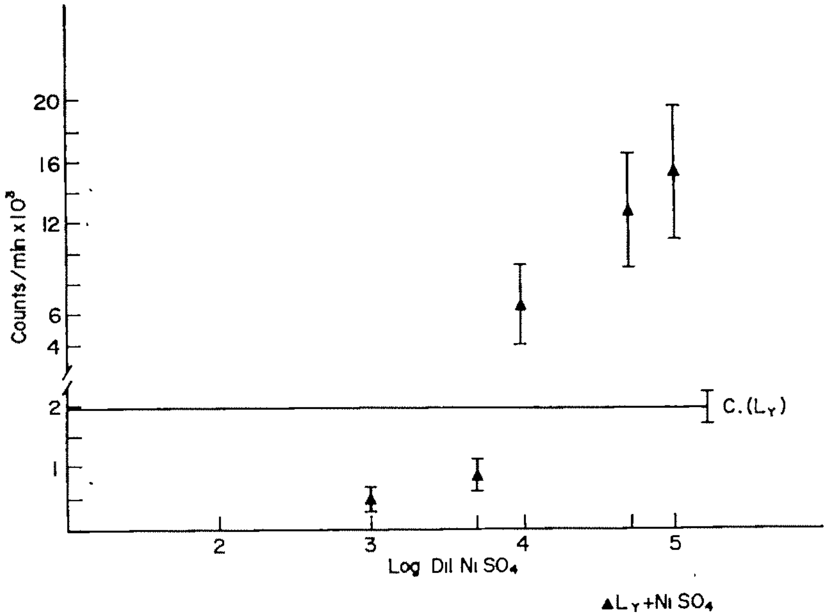

FIGURE 2. Mean and standard deviations of thymidine incorporation in the LTT in fourteen nickel sensitive patients with serial dilutions of $NiSO_4$ in the lymphocyte culture. 'High dilution responders'.

TABLE 3. LTT in eleven nickel sensitive patients with serial dilutions of $NiSO_4$ in the lymphocyte culture. 'Low dilutions responders', the maximal incorporation of thymidine occurring at the lowest dilutions of $NiSO_4$

Cases	Counts per minute						
	Tube control	Lymphs. P.H.A.	$NiSO_4$ Dilutions				
			1:1000	1:5000	1:10,000	1:50,000	1:100,000
I	2297	37,620	5727	1328	1393	890	527
II	983	111,486	3509	1471	1530	580	579
III	1880	54,289	6693	4763	3629	1112	763
IV	1880	45,268	8124	6123	3520	890	627
V	3787	168,350	10,211	10,357	13,775	4227	4535
VI	1959	98,126	10,520	9627	8512	1116	960
VII	1861	142,601	3942	6756	5778	3417	2269
VIII	716	13,299	2527	2118	2175	2217	2021
IX	1081	39,216	4188	2710	2292	2167	1831
X	690	69,345	4889	3455	2128	1827	1716
XI	717	59,428	—	1389	2269	2273	1498
Mean	1622	76,274	6033	4554	4273	1883	1575
Standard deviation	280	14,524	884	987	1145	344	351

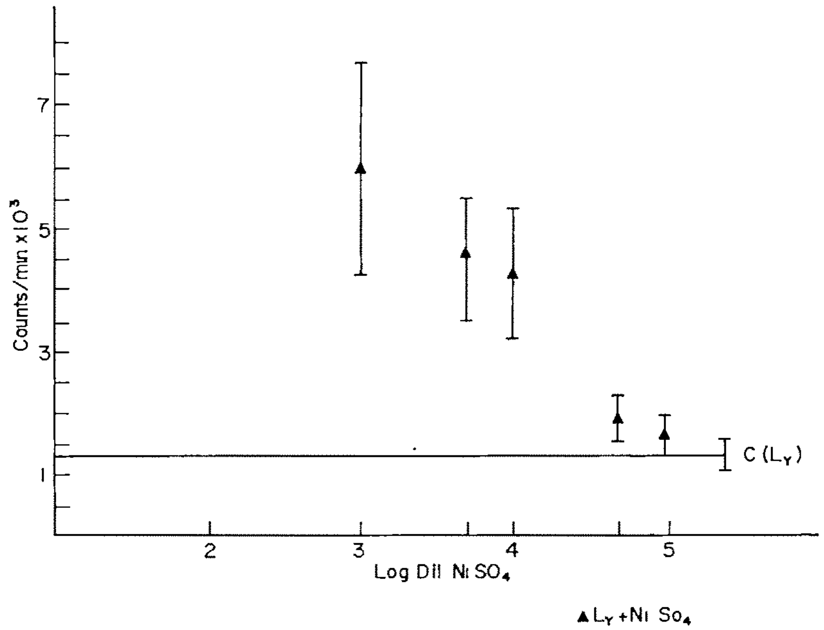

FIGURE 3. Mean and standard deviations of thymidine incorporation in the LTT in eleven nickel sensitive patients with serial dilutions of $NiSO_4$ in the lymphocyte culture. 'Low dilution responders'.

and 763 cpm at 1:100,000. This group of patients could be considered 'low dilution responders'. A graph of the results obtained appears in Fig. 3.

DISCUSSION

In our experience lymphocytes from control donors failed to transform when incubated with serial dilutions of $NiSO_4$. The only effect which could be attributed to the presence of $NiSO_4$ in the culture medium was a slight decrease in uptake after incubation with tritiated thymidine, suggesting a toxic effect of $NiSO_4$ on the incubated lymphocytes. In this respect it is difficult to compare our results with those obtained by other workers. Each used different techniques for the LTT, employing leukocytes or lymphocytes and nickel sulphate or acetate at constant dilution. However, when no blast transformation occurred in control donors with $NiSO_4$ our results are in agreement with those of MacLeod, Hutchinson & Raffle (1970), Forman & Alexander (1972), and Hutchinson *et al.* (1972). These authors used nickel sulphate as antigen in their lymphocyte culture. We did not find the non-specific transformation of lymphocytes induced by nickel as postulated by Aspergren & Rorsman (1962), and Pappas *et al.* (1970). These authors used nickel acetate as antigen. Interpretation of the conflicting results obtained should take into account the different nickel salts used in their experiments.

All of our twenty-five patients with positive patch test nickel dermatitis demonstrated a positive LTT when $NiSO_4$ was present in serial dilutions in the lymphocyte culture medium. We observed two types of response, depending on the dilution of $NiSO_4$. In the group of 'high dilution responders' (fourteen patients) lymphocyte blast transformation was highest at the lowest concentration of $NiSO_4$. In nine of these cases the LTT remained negative at dilutions of 1:1000 and 1:10,000. If higher dilutions had not been employed, the nine patients would have been considered LTT negative.

In the group of 'low dilution responders' (eleven patients) the LTT was more reactive when $NiSO_4$ was present in higher (1:1000–1:5000) concentrations.

Hutchinson *et al.* (1972) used a 10^{-4} mEq/mol concentration of nickel in the culture medium for the LTT. They did not use serial dilutions. The results in two of their patients (Nos. vii and viii) approximated the controls at the given concentration of $NiSO_4$. These two patients may be comparable with our second group of responders where LTT was strongly reactive only at the higher concentrations of $NiSO_4$.

We could not find clinically significant differences between the two groups. All patients in the study had had their dermatitis for several years, and the age and sex of each group were approximately the same. The LTT was carried out at a time when their dermatitis was still clinically active.

In our experience the presence of autologous plasma in the LTT is not necessary. We did not use it in our experiment and transformation of lymphocytes took place in the presence of nickel sulphate dilutions in a consistent manner. In Forman & Alexander's (1972) experiment, transformation of lymphocytes occurred only in the presence of autologous plasma.

The nature of the antigen responsible for the reaction remains unknown. Direct conjugation of a simple chemical with a protein component of the lymphocyte cell membrane has been known to occur in dinitrofluorobenzene contact dermatitis in guinea-pigs, as reported by Geczy & Baumgarten (1970).

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Atopic disease and serum immunoglobulin-E

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SUMMARY

Fifty-seven subjects, forty-three with various combinations of atopic disease and fourteen non-atopic controls, were studied using a battery of immediate skin test allergens and a radio-immunoassay for serum Immunoglobulin-E (IgE).

The geometric mean serum IgE level (units/ml) was 50 in strictly nonatopic controls, 170 in atopic respiratory disease (ARD) patients, 320 in atopic dermatitis (AD) patients and 772 in those patients with both AD–ARD. The marked elevation of the serum IgE in the latter group was not associated with respiratory disease activity and not closely correlated with extent of the dermatitis, but may relate to patients with both AD–ARD being 'highly atopic'.

Overall, there was no correlation between the serum IgE level and the frequency of positive skin tests. Compared to ARD patients, the serum of AD patients contained more IgE, yet they reacted significantly less frequently to common extrinsic allergens.

INTRODUCTION

Several publications have reported that the highest serum levels of Immunoglobulin-E are found in individuals with atopic dermatitis, many of whom also have asthma or allergic rhinitis, or both (Juhlin *et al.*, 1969; Ogawa *et al.*, 1971; Kumar *et al.*, 1971a; Kumar, Newcomb & Hornbrook, 1971b; Gurevitch, Heiner & Resiner, 1973). This study examines the relationship between the IgE level and immediate allergen skin test sensitivity in non-atopic controls and atopic individuals with different clinical manifestations, with emphasis on atopic dermatitis.

MATERIALS AND METHODS

The patients were either Army active-duty personnel or their dependants from the clinics of Letterman Army Medical Center. When the clinical history, medical record and physical examination revealed only cutaneous manifestations without personal evidence of allergic respiratory disease (ARD), that patient was classified as having atopic dermatitis (AD). The criteria used to diagnose atopic dermatitis

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have been detailed in a separate publication (Jones, Lewis & McMarlin, 1973), but it should be repeated that only typical cases were accepted for study. Patients with a documented history of allergic rhinitis (AR) or allergic bronchial asthma (BA) or both, who did not also have AD, were classified as having ARD. Those patients with either a history of or with active ARD who also had AD were classified as having co-existent AD–ARD. Patients taking systemic medications or undergoing hyposensitization therapy were excluded to avoid potential interference with IgE levels or skin test results.

A dietetic intern group was solicited for volunteers and fourteen age- and sex-matched healthy non-atopic individuals were selected for controls. Informed consent was obtained after the nature of the procedure had been fully explained. The clinical characteristics of the four study groups are presented in Table 1.

TABLE 1. Clinical characteristics of the groups

Clinical group	No.	Mean age (years)	Extremes of age (years)	Mean age of onset (years)		
				AD	BA	AR
Atopic dermatitis (AD)	14	19 4	11–41	6·2	0	0
Allergic respiratory disease (ARD)	12	24·8	21–37	0	10 1* ($n = 6$)	12·0* ($n = 10$)
Co-existent AD–ARD	17	20·9	15–38	5·0	5·5† ($n = 9$)	11·3† ($n = 14$)
Non-atopic controls	14	23·2	19–27	0	0	0

* Four subjects had both BA–AR.

† Six subjects had both BA–AR.

Immediate allergen tests

Ten antigens or antigen mixtures, frequently eliciting immediate allergic reactions at the Letterman Allergy Clinic (see list in Table 3), were used for scratch tests on the upper mid-back. Subsequently, each antigen to which a subject failed to react was given intradermally on the posterior deltoid skin. Both scratch and intradermal tests were interpreted at 20 min and positive tests were those which gave a 2-plus or greater weal and flare reaction, after subtracting the response to the control solution. The skin tests were administered immediately following withdrawal of the blood sample. The peak pollen season in San Francisco is during May and June, so the study was conducted during the interval from September to December to minimize seasonal variations.

Immunoglobulin-E assay

Blood samples were allowed to clot at room temperature, centrifuged and the serum aseptically removed and stored at −20° or −70° C until determinations were made. The assays were performed using Phadebas IgE radio-immunoassay kits (Pharmacia). Multiple determinations of standards over a 1 year period showed excellent agreement; the coefficient of variation ranged from 1·7 for the low level standard to 3·6 for the high level standard. Laboratory standards and unknown serum samples were exchanged with Richard Newcomb, Denver, who used Rowe's modified Mancini technique; and with Michael Bazaral, San Diego, who used a bromacetylcellulose-based radio-immunoassay technique. The three procedures agreed within the limits of experimental error. The IgE levels are reported in units per ml as recommended by Pharmacia. The units corresponded numerically to the nanogram values obtained by Newcomb (Newcomb & Nelson, 1973) but not to Bazaral's nanogram values, unless they were converted to units by multiplying by 2·4.

RESULTS

Each individual's IgE level is plotted on the log graph in Fig. 1. The mean IgE level was lowest in the controls, and progressively more elevated through the ARD, AD and AD–ARD groups. An analysis of the data presented in Fig. 1 using the Kruskal–Wallis method reveals an H value of 27·51 compared at three degrees of freedom to a critical χ^2 value of 7·81. Thus, there is sufficient evidence ($P<0\cdot05$) to reject the null hypothesis that the four groups have identical distributions of serum IgE.

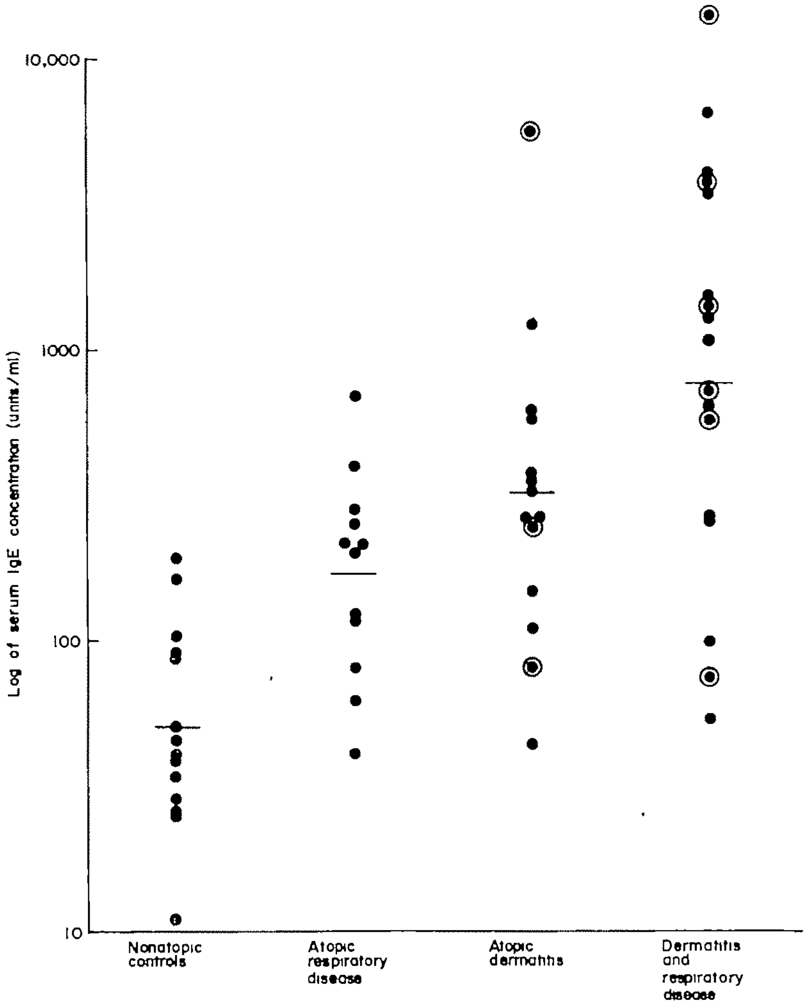

FIGURE 1. Each dot represents the serum IgE level for one subject. The geometric mean IgE levels for each group are indicated by horizontal bars. Encircled dots indicate that subject had extensive atopic dermatitis.

Further analysis using Dunn's Rank Sum procedure for multiple comparisons and assuming an experimental error of 0·05 reveals that: (1) the IgE levels of the AD and ARD–AD groups differ from those of the nonatopic control group; (2) there is not sufficient evidence (with this error control) to indicate that any other two groups' serum IgE differ one from the other.

The severity of the skin disease as reflected in extent of involvement was graded to elucidate whether the IgE level was related to the area of skin involved. The thirty-one AD patients were assigned to one of four subgroups depending on the extent of their skin lesions.

1. *Extensive.* Generalized, or involvement of at least five of the anatomical areas listed below.
2. *Average.* Involvement limited to three or four of the following areas: antecubital fossae, popliteal fossae, neck, face, forearms, hands.
3. *Mild.* Involvement limited to two of the anatomical areas listed above.
4. *Temporarily generalized.* Involvement similar to the average group, except each had experienced at least one episode of generalized involvement. The IgE results per subgroups are given in Table 2.

TABLE 2. Extent of skin disease and IgE level

Severity of skin disease	No. of subjects	Geometric mean IgE level and extremes	
		Mean	Min. Max.
Extensive	9	873	76–14,750
Temporarily generalized	8	569	45– 6,650
Average	11	458	53– 3,400
Mild	3	288	150– 600

Analysis for any effect the severity of the dermatitis had on the IgE level was made using the Jonckheere method. The J value is found to be 1·02 compared to a critical J value of 1·645. Thus, there is not sufficient evidence that the IgE levels of the subgroups differ at the $P<0{\cdot}05$ level.

Table 3 summarizes the skin test and IgE results for the atopic subgroups and controls. Analysis of the frequency of positive skin tests by the χ^2 test revealed a value of 33·68 indicating that the number of responses was not the same for the four clinical groups ($P<0{\cdot}05$). Multiple positive skin tests occurred most frequently in the ARD group. Further analysis using the Bresnahan and Shapiro technique revealed that: (1) there was no significant difference in the frequency of positive tests

TABLE 3. Immediate skin test responses* and IgE levels

Clinical group	No. of subjects	Number of positive skin tests			Geometric mean serum IgE level
		0	1—2	3 or more	
AD	14	9	4	1	320
ARD	12	1	1	10	170
AD–ARD	17	0	7	10	772
Non-atopic controls	14	9	4	1	50

* 1. Mixed Trees; 2. Mixed Grasses; 3. Plantain; 4. Ragweed; 5. Mixed Weeds; 6. House Dust; 7. Gp. #1 Moulds; 8. Egg; 9. Milk; 10. Wheat; 11. Control.

between the nonatopic controls and the AD group ($P>0.05$); (2) there was a striking difference ($P<0.05$) in the distribution of responses between those subjects having ARD (ARD and AD–ARD groups combined) and those not having ARD (non-atopic controls and AD groups); (3) within the combined ARD group there is a suggestion ($\chi^2 = 3.73$, df = 1, $P<0.10$) that the patients having only ARD more frequently had three or more positive tests.

There was no apparent relationship between the frequency of positive skin tests and the serum IgE level when all fifty-seven subjects were compared. Only within the AD–ARD subgroup (seventeen subjects) was there a suggestion that there might be an association between the number of positive allergen tests and the quantity of IgE in the serum. The association was not significant at this sample size. There was no evidence that concurrent activity of ARD was related to the concentration of IgE in the serum of the seventeen AD–ARD patients.

DISCUSSION

The serum IgE is obviously elevated in some atopic subjects (Juhlin *et al.*, 1969; Ogawa *et al.*, 1971; Kumar *et al.*, 1971a; Kumar *et al.*, 1971b; Gurevitch *et al.*, 1973) and in certain other non-atopic conditions (Heiner & Rose, 1970). It is difficult to ascertain what the upper limits of normal should be. Probably the best study of this problem was made by Johansson (1968) who, based on a statistical sample of the Swedish population and a random sampling of blood donors, determined the mean serum level to be 248 ng/ml with the upper confidence at 1000 ng/ml.

The upper limit for the serum IgE concentration in healthy non-atopic individuals has been studied by several investigators. Using a sensitive technique, Bazaral, Orgel & Hamburger (1971) found infant cord serum to contain 2·1 units/ml, and to increase slowly to 57·6 units by 6 months. Bazaral *et al.* (1971) found postpartum mothers to have a serum IgE level of 205, but he noted that some had an allergic history. Ogawa (1971) studied twenty-eight normal non-atopic subjects and found a mean serum level of 143 ng/ml. Gleich, Auerbach & Swedlund (1971) found their normal non-atopic controls to have a mean serum level of 179 with 95% confidence limits extending up to 780 ng/ml. Spitz *et al.* (1972) apparently used more rigorous criteria for selection of non-atopic controls for they found a mean IgE level of 88 ng/ml. The fourteen strictly non-atopic controls reported herein had a geometric mean level of 50 units/ml serum. These individuals were selected because they denied having had symptoms or signs suggestive of AD or ARD. Even some members of this carefully chosen group had positive weal and flare skin test reactions to extrinsic allergens (Table 3). It would appear then, that the upper limit of serum IgE in non-atopic individuals is lower than previously thought. Further studies will be necessary to define the normal levels of IgE in strictly non-atopic individuals.

Our twelve patients with only ARD (2, BA; 6, AR; 4, BA–AR) had a geometric mean serum IgE of 170 units/ml. The serum IgE level has been documented to be elevated in patients with ARD (Kumar *et al.*, 1971a; Kumar *et al.*, 1971b; Gleich *et al.*, 1971; Berg & Johansson, 1969). These authors noted that the highest IgE levels were in ARD patients (mostly BA or both BA–AR) who also had AD. On the other hand, it is difficult to ascertain from these reports what the serum IgE was in those ARD patients in whom there was neither current evidence for nor a past history suggestive of AD. Our data, which admittedly is based on a small patient group, suggests that many patients with ARD (either or both BA and AR) who do not have AD or a history thereof have normal to moderately elevated serum IgE levels.

Of the numerous studies of IgE in atopic disease, only a few placed special emphasis on AD. Yet, all have reported the concentration of this immunoglobulin to be elevated in this chronic skin disease of unknown etiology. The mean serum level was found by Ogawa *et al.* (1971) to be 493 units/ml in thirty-eight patients; by Gurevitch *et al.* (1973) to be 1178 units/ml in forty-eight patients; by

P, 25, 463

Clendenning *et al.* (1973) to range from 100 to 960 units/ml in different study groups; by Juhlin *et al.* (1969) to be 2733 ng/ml in twenty-eight patients; and by Stone, Muller & Gleich (1973) to be 3067 ng/ml in fifty-eight patients. Although the mean for each AD group reported is unquestionably elevated, there have been remarkable differences in the individual patients' IgE level. Since AD patients differ from one to another in clinical manifestations and associated atopic conditions, some variation in the IgE level may be related to the associated clinical parameters. Evidence suggests that the most important clinical factors to consider are (1) severity or extent of skin disease and (2) presence of coexistent ARD.

The first possibility has been considered extensively. Juhlin *et al.* (1969) found no relationship between severity of AD and the IgE level, but Ogawa *et al.* (1971) reported a strong correlation between IgE level and severity of skin disease. If severity is equated with extent of skin involvement, it appears that Gurevitch *et al.* (1973), Clendenning *et al.* (1973) and Stone *et al.* (1973) are in substantial agreement with Ogawa.

We also examined the relationship between serum IgE level and extent of dermatitis in thirty-one AD patients (fourteen AD only, seventeen AD–ARD). Comparing the mean IgE levels of the sub-groups revealed a trend for extensive atopic dermatitis to be associated with the higher serum IgE levels (Table 2). Although this apparent trend is consistent with the correlation mentioned above, between the extent of AD and the concentration of serum IgE, the differences between the distribution of IgE levels in these sub-groups based on extent of disease were not significant. A more direct comparison of the serum IgE and severity of dermatitis may be had by examination of Fig. 1. Of the eleven patients with IgE levels greater than 1000 units/ml, only four were classified as having extensive dermatitis. Conversely, five of the nine patients with extensive dermatitis had levels less than 1000 units/ml. In fact, two of these five had less than 100 units/ml. Thus, in this study the extent of the dermatitis was not closely associated with the serum IgE concentration.

The second clinical variable which may influence the AD patient's serum IgE level is presence or absence of coexistent ARD. Juhlin *et al.* (1969), Clendenning *et al.* (1973), and Stone *et al.* (1973) found no statistically significant correlation between the AD patients' IgE level and the presence of coexistent ARD. Juhlin's group reinvestigated the 'pure' AD cases and found that many of the patients suffered from sub-clinical atopic respiratory symptoms, hence their initial conclusion would no longer be valid (Johansson, Bennich & Berg, 1972). The data presented by Ogawa *et al.* (1971) strongly suggests that the IgE levels were lower in AD patiehts that did not have ARD, however, they emphasized a positive correlation between severity of the dermatitis and elevated IgE. More recently, Johnson *et al.* (1974) reported in an abstract that the serum IgE level of AD patients was significantly associated with both severity of AD and coexistence of ARD.

In this study, the AD patients who denied having ARD had a geometric mean serum IgE of 320 units/ml. In contrast, the mean serum IgE of the AD–ARD patients was 772 units/ml (Fig. 1 and Table 3). Of the seventeen patients in the AD–ARD group, nine had levels above 1000. In fact, considering all fifty-seven subjects, nine of the eleven with levels above 1000 were members of the AD–ARD group. Of the patients with solely AD, only two had levels greater than 1000, whereas twelve were within the normal range for several laboratories whose work has been extensively quoted herein. Thus, this study confirms the preliminary report of Johnson *et al.* (1974) and indicates that many, if not most, adult patients who have AD but no ARD will be found to have only moderately increased serum IgE concentrations. Clendenning *et al.* (1973) have in fact reported that more than 50% of their series of AD patients had levels below 700, despite the fact that some had ARD.

Thus, a definitive statement regarding the specific correlate(s) of elevated IgE in AD patients cannot be made at this time. It seems safe to expect the serum IgE to be elevated if there is extensive AD and/or coexistent ARD. A simple, although speculative, explanation for these clinical associations would be that such patients are genetically 'highly atopic', and consequently have an enhanced ability for the biosynthesis of IgE. This would be consistent with recent evidence which suggests that the basal IgE level is controlled by simple Mendelian genetics involving the segregation of two alleles at a single locus (Bazaral *et al.*, 1971; Opree, Krause & Stockberg, 1972). Although this hypothesis may help explain immunoglobulin levels, it does not account for the diverse clinical expression of the atopic state.

Skin tests, serum IgE and clinical manifestations

Comparing the results from all fifty-seven subjects, there was no correlation between the number of positive skin tests and the IgE level (Table 3). From that same table it can be seen there was a strong correlation between multiple positive tests and ARD. Coca & Cooke (1923), in defining the term 'atopy', emphasized this close association. Both the ARD and AD–ARD groups exhibited multiple positive skin tests.

In contrast, the AD group reacted to the ten allergen test battery less frequently than the ARD ±AD) patients. In fact, the frequency of response of the AD group was not significantly greater than that of the non-atopic controls. This confirms the earlier reports which showed that patients with typical AD (no ARD) often do not show multiple positive immediate skin test reactions to extrinsic allergens (Benekens-Kestelyn, 1965; von Schnyder, 1958; Rajka, 1961). In this regard, the finding of moderately to extremely elevated IgE levels in some AD patients is particularly interesting. Stone *et al.* (1973) found a correlation between the atopic index (percentage of allergen tests that were

TABLE 4. Atopic dermatitis: Clinical features, immediate allergy and IgE

No. of subject	Age (years)	Sex	Race	Age at onset (years)	Predominant involvement	Severity	Positive immediate allergy skin tests	IgE units/ml	Family history	
									AD	ARD
1	22	F	C	Infantile	Hands and leg	T	1	350	2–S 1–P	0
2	23	F	C	10	Hands and feet	M	0	600	1–S	0
3	18	F	O	16	Flexures, face, neck, ears	A	0	263	1–S 1–P	0
4	16	F	O	5	Flexures, neck, face	A	0	580	1–S 1–P	0
5	15	F	B	Infantile	Flexures, neck, hands	A	8	380	1–S 1–P	1–S
6	41	F	B	13	Hands, back	M	0	150	2–C	1–C
7	20	F	B	5	Hands and legs	T	0	45	0	1–P
8	18	M	C	Infantile	Flexures, neck, hands	A	2	110	0	1–S
9	20	F	C	Infantile	Flexures, neck, hands	A	2	260	0	1–P
10	15	F	C	Infantile	Flexures and hands	T	1	320	0	0
11	20	F	C	19	Generalized	E	0	80	1–P 1–S	1–P
12	11	M	C	Infantile	Generalized	E	0	250	0	0
13	14	F	C	Infantile	Flexures and hands	T	0	1200	0	0
14	18	F	B	14	Generalized	E	0	5750	0	0

B–Black; C–Caucasian; O–Oriental.

Severity: E–Extensive; T–Temporarily generalized; A–Average; M–Mild; AD–Atopic Dermatitis; ARD–Atopic Respiratory Disease; S–Sibling; P–Parent; C–Children.

positive) and the serum IgE level, but it should be noted that most of their patients had ARD. Clendenning *et al.* (1973) noted that the mean IgE level of five AD patients with no positive allergen skin tests was 1616 units/ml. In our fourteen patients with solely AD (Table 4), there was no relationship between frequency of skin test positivity and IgE level. In fact, the two patients having the highest levels did not react to any test and the patient exhibiting the most positives (eight) had an IgE level of only 380 units/ml. This apparent inconsistency deserves further examination.

An immediate skin test reaction to an allergen reflects that antibody (presumably IgE), specific for that allergen, is fixed to tissue mast cells (Ishizaka & Ishizaka, 1973). ARD patients and even some non-atopic individuals raise and fix specific IgE in their skin and when challenged with that specific antigen manifest an immediate skin test reaction. This occurs even though the amount of circulating IgE may be relatively small, particularly in the non-atopic individuals. In contrast, many patients with solely AD have considerably more circulating IgE, yet react no more frequently than non-atopic subjects. In fact, comparing the AD patients to the non-atopic controls reveals a mean IgE level in the former of more than six times the latter, yet the frequency of skin test reactions was similar. This suggests that individuals with solely AD may fail: (1) to synthesize IgE specific for common extrinsic allergens; (2) to fix the resulting antibody to skin mast cells; (3) to release the mediators of Type I reactions following specific antigen challenge. The discordancy in AD patients between the frequency of positive skin tests and IgE levels is particularly interesting.

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The interaction of Concanavalin A and the surface coat of stratified squamous epithelium

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SUMMARY

The interaction of Concanavalin A (Con A) and the surface membranes of the epithelial cells of human oral mucosa, epidermis and guinea-pig lip was studied. Two methods were used: fluorescent Con A and Con A in combination with horse radish peroxidase. With both methods the surface coat of epithelial cells was stained. The specificity of the staining methods could be demonstrated. The interaction between pemphigus antibodies and the surface membranes of epithelial cells could be blocked by Con A.

INTRODUCTION

Anti-epithelial antibodies may be detected in a number of clinical conditions, especially pemphigus (Beutner, Chorzelski & Jordon, 1970) and also in some cases of extensive burns (Ablin *et al.*, 1969; Leguit *et al.*, 1973; Thivolet, Beyvin & André, 1970), toxicoderma (Fellner *et al.*, 1973), benign prostate hypertrophy and prostatic carcinoma (Ablin, 1973a, b). In pemphigus much is known about these antibodies, which are directed against a substance localized in the intercellular areas and on the cell membranes of stratified squamous epithelium (Beutner *et al.*, 1970; Chorzelski *et al.*, 1968).

It is possible to produce tissue specific pemphigus-like antibodies in rabbits by immunization with crude epithelial extracts (Ablin, 1969; Grob & Inderbitzin, 1967a). In contrast to this little is known about the antigen(s) involved in the production of these antibodies. Efforts to isolate and characterize the antigen had only poor results (Ablin & Beutner, 1968). Some authors suggest that these antigens may be glycoproteins localized in the intercellular areas, or in the surface coat of the epidermal cells (Beutner & Shu, 1973; Grob & Inderbitzin, 1967a; Wolff, 1971). There are several methods for the histochemical demonstration of polysaccharides in the surface coat of epithelial cells (Mercer, Jahn & Maibach, 1968; Wolff & Schreiner, 1968).

In this paper we report on the interaction of the lectin Concanavalin A (Con A) with glycoproteins in the surface coat of stratified squamous epithelium. Con A reacts specifically with a variety of polysaccharides and glycoproteins containing α-D-glucopyranosyl or α-D-mannopyranosyl residues at their non-reducing chain endings. This interaction can be specifically inhibited by some low molecular weight carbohydrates, the most potent inhibitor being α-methyl-D-mannoside (Goldstein, Hollerman & Smith, 1965). In this study we used two methods: Con A conjugated with fluorescein isothiocyanate (Con A-FITC), and Con A in combination with horse radish peroxidase (Con A-HRP). Furthermore, we investigated the possible identity between the Con A receptor sites and the pemphigus antigenic sites on the cell walls.

MATERIALS AND METHODS

Punch biopsies of normal human skin (3 mm diam.) and excision biopsies of normal human oral mucosa* and guinea-pig lips were quick frozen in liquid nitrogen. All experiments were done on unfixed as well as on acetone fixed (10 min at 4°C) 4μ cryostat sections.

Concanavalin A, diaminobenzidine (DAB), sodium periodate, and α-methyl-D-mannoside (MEM) were obtained from Sigma Chem. Co. Horse radish peroxidase, grade II (HRP), was obtained from Boehringer, Mannheim; Fluorescein isothiocyanate, isomere I (FITC) was obtained from BBL Comp. Div. Bio Quest. Fluorescein conjugated horse anti-rabbit Ig (HoARaIg-FITC) and conjugated horse anti-human Ig (HoAHuIg-FITC) were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam). Rabbit anti-bovine oesophageal epithelium serum was prepared as described below. All solutions were in phosphate buffered saline, pH 7·2 (PBS) unless otherwise stated.

Con A-FITC conjugate method

Con A was conjugated with FITC according to Smith & Hollers (1970). The reaction product was freed from unconjugated FITC using a Sephadex G 25 column; thereafter the conjugate was cleared by centrifugation. The characteristics of the conjugate were: protein content 1 mg protein per ml, molar fluorescein/dimer ratio 1·2, mean molar fluorescein/protein ratio 1·8 (see Van Lis & Kalsbeek, 1973). Cryostat sections were incubated with Con A-FITC (1:150) for 10 min at room temperature. After washing with PBS (3 × 5 min) the stained sections were mounted in Bacto Fa Mounting Fluid (Difco Lab) and examined with an UV fluorescence microscope (see Van Lis and Kalsbeek, 1973).

Con A-HRP method

The method as described by Bernhard & Avrameas (1971) was followed. The sections were treated with 0·5 mg Con A per ml PBS for 20 min, followed by incubation with HRP (1 mg/ml). Thereafter the sections were stained according to Graham & Karnovsky (1966) with DAB (5 mg in 10 ml 0·05 M Tris-HCl buffer pH 7·6 and 0·05 ml 3% H_2O_2) for 5 s at room temperature. After each step the preparations were washed with PBS (3 × 5 min).

Finally, the sections were dehydrated in ethanol and xylene, mounted in malinol and examined with a Zeiss light microscope.

Processing for electron microscopy

Unfixed 40μ cryostat sections of oral mucosa were washed in 0·15 M sodium acetate pH 5·5. They were next incubated with Con A (10 mg/ml) in the same buffer for 24 h at 4°C. After rinsing with PBS for 1½ h, incubation followed with HRP (1 mg/ml PBS). The preparations were rinsed with PBS for 1 h and the diaminobenzidine reaction was performed as described above.

Then the pieces were rinsed with PBS and post-fixed in osmium tetroxide according to Millonig (1961) at room temperature. After dehydration in ethanol the preparations were embedded in epon. The uncontrasted ultrathin sections were examined with a Zeiss 9A electron microscope (Dept. of Electron Microscopy, Free University, Amsterdam, head: Dr E.C.M.Hoefsmit).

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*Obtained from the Dept of Oral Surgery of the Free University (head: Professor Dr W.A.M.van der Kwast).

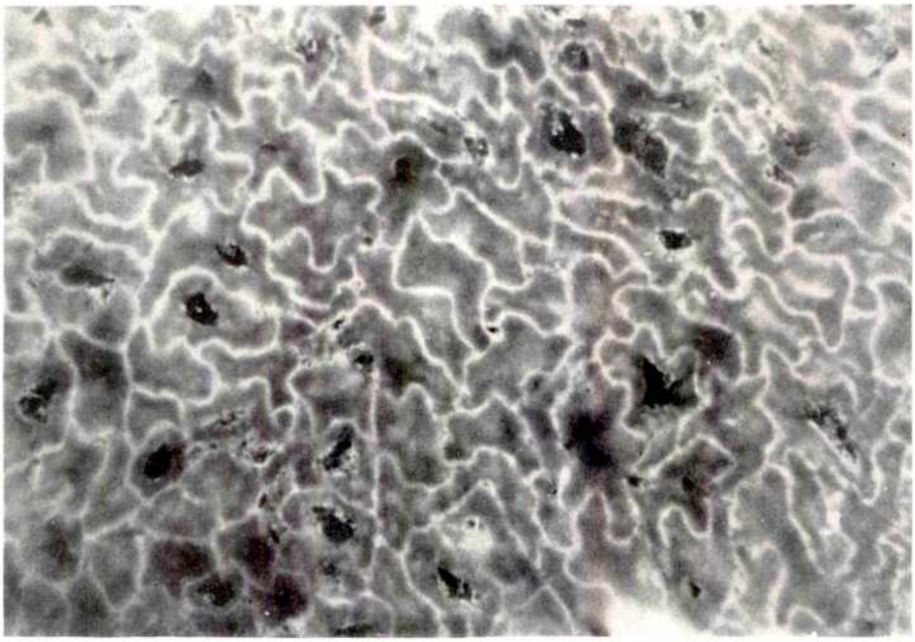

FIGURE 1. Staining of human oral mucosa with Con A-FITC. Note the brilliant staining of the cell walls (× 2000).

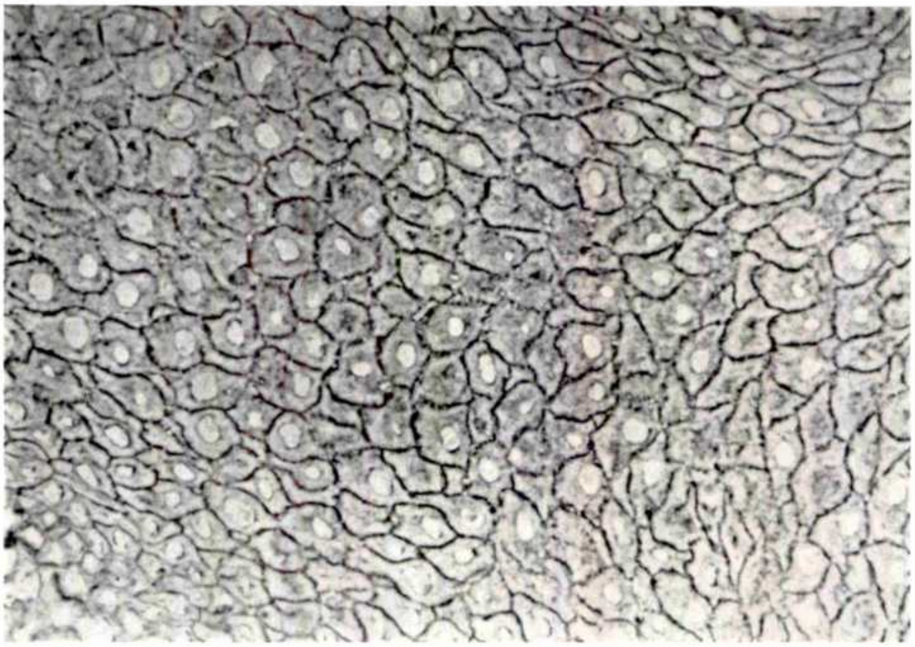

FIGURE 2. Staining of human oral mucosa using the Con A-HRP method. The cell walls show a linear, partly granular staining pattern. (× 1300).

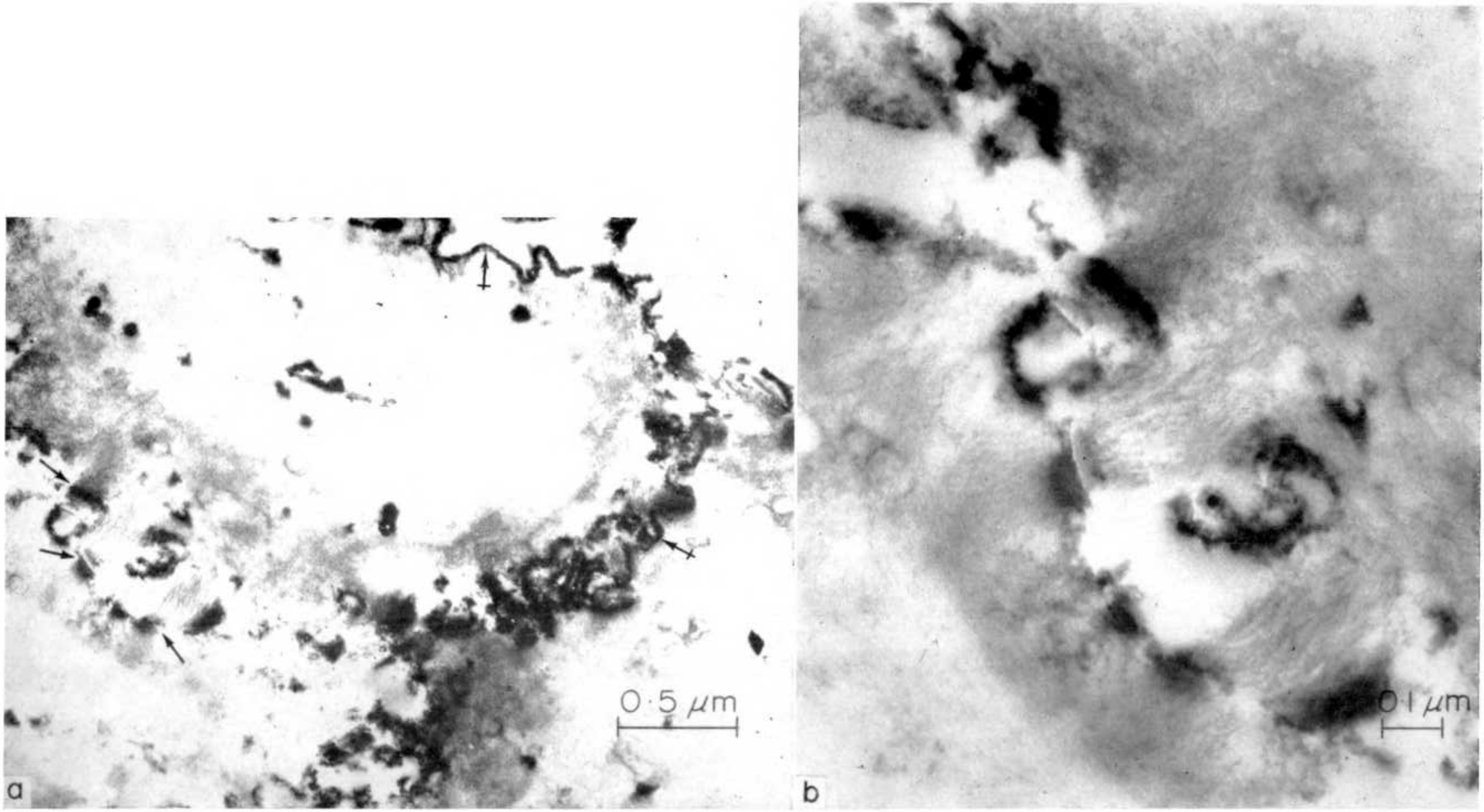

FIGURE 3. (a) Electron microscopy of human oral epithelium after staining with the Con A-HRP method. Note the heavy deposits of the reaction product in the cell membranes (+→) and in the region of the desmosomes (⟶) (× 36,000); (b) Detail of the desmosomes (× 64,800).

Control and specificity tests

Control and specificity tests were done mainly on unfixed cryostat sections of oral mucosa, using unlabelled Con A (2 mg/ml); 0·25 M MEM in 0·05 M phosphate buffer pH 7·2 and 0·002 M sodium periodate in 0·2 M phosphate buffer pH 7·2. Furthermore sections were stained with a fluorescein isothiocyanate solution (1 mg/ml, diluted 1:10,000).

Preparation of rabbit anti-epithelium serum

Crude bovine oesophageal epithelium antigen was prepared according to Grob & Inderbitzin (1967a). Five rabbits were immunized four times by intramuscular injections, at 2-weekly intervals. The animals were bled 2 months after the final injection. Two of the rabbits showed circulating antibodies directed against cell boundaries of epithelial cells of human skin, human oral mucosa, and guinea-pig lip. The immunofluorescence titre of both sera as determined by the indirect immunofluorescence test (IF-test) was 1:80.

RESULTS

Staining results

After treatment with Con A-FITC, as well as with Con A-HRP, the cell walls of the epithelial cells of human mucosa, human epidermis and of guinea-pig lip were stained (Figs. 1 and 2).

The intercellular bridges were stained the most intensively. The epithelial cell boundaries of the oral mucosa and the guinea-pig lip were stained more brilliantly than those of the human epidermis. The surface membranes of the basal cells seemed to be less reactive than those of the prickle cells of the stratum spinosum. The cytoplasm of the epithelial cells showed a slight background staining.

Electron microscopy of human oral mucosa, using Con A-HRP, showed an intensive staining of the cell membranes and the desmosomes (Fig. 3).

Control and Specificity tests

The results of the control and specificity tests are summarized in Tables 1 and 2.

The staining of the cell walls could be inhibited by pre-treatment with unlabelled Con A, MEM

TABLE 1. Control and specificity tests of the Con A-FITC staining method on sections of human oral mucosa

Treatment, pre-treatment and post-treatment	Fluorescent staining of epithelial cell membranes
Staining with Con A-FITC (1:150, 10 min)	+
Staining with FITC solution (1 mg/ml, 1:10,000 diluted)	−
Pre-treatment with Con A (2 mg/ml, 20 min), followed by staining with Con A-FITC (10 min)	±/−
Staining with Con A-FITC plus Con A (2 mg/ml, 10 min)	±/−
Staining with Con A-FITC plus methyl mannoside (0.25 M in PBS, 10 min)	±
Staining with Con A-FITC (10 min), followed by post-treatment with methyl mannoside (0.25 M in PBS, 1 h)	−
Staining with Con A-FITC (10 min), followed by post-treatment with PBS (1 h)	+
Pre-treatment with periodate (0.002 M in PBS, 3 h), followed by staining with Con A-FITC (10 min)	−
Pre-treatment with PBS (3 h), followed by staining with Con A-FITC (10 min)	+

TABLE 2. Control and specificity tests of the Con A-HRP method on sections of human oral mucosa

Treatment, pre-treatment and post-treatment	Staining of epithelial cell membranes
Staining with the Con A-HRP method	+
Staining with Con A-HRP, with omission of Con A	−
Staining with Con A-HRP with omission of HRP	−
Treatment with Con A plus methyl mannoside (0.25 M in PBS, 10 min), followed by HRP	±
Treatment with Con A, followed by post-treatment with methyl mannoside (0.25 M in PBS, 30 min), thereafter staining with HRP	−
Treatment with Con A, followed by post-treatment with PBS (30 min), thereafter staining with HRP	+
Pre-treatment with periodate (0.002 M in PBS, 1 h), followed by staining with the Con A-HRP method	−
Pre-treatment with PBS (1 h), followed by staining with the Con A-HRP method	+

and periodate respectively (see Fig. 4). After staining with a FITC solution no fluorescence of the cell walls was observed.

Effect of Fixation

The effect of several fixations on the staining of the cell membranes of human oral mucosa was investigated. After fixation with ethanol (96%) and methanol the staining was greatly diminished.

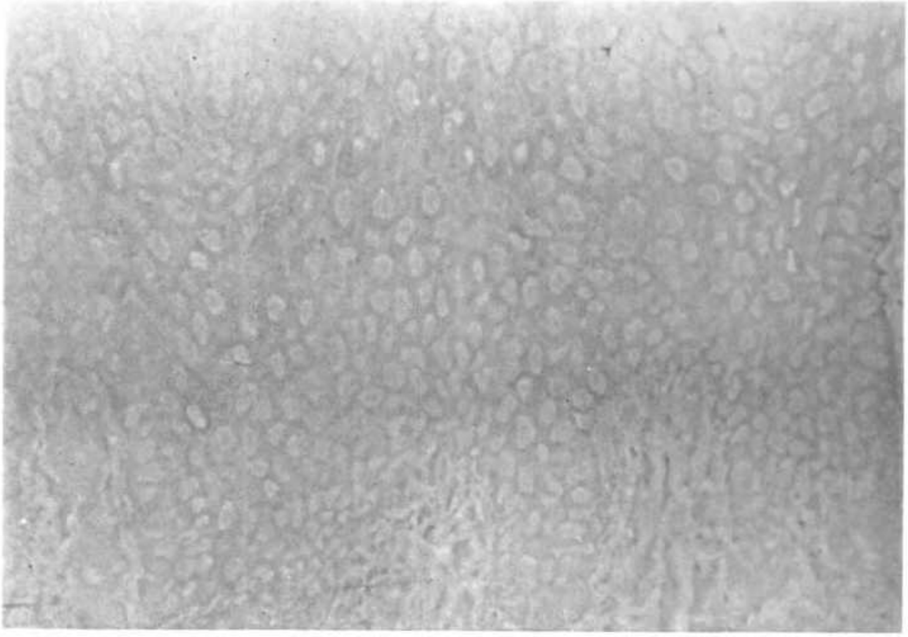

FIGURE 4. Control of the Con A-HRP method. After post-treatment with methyl mannoside the staining of the cell boundaries is absent (× 1300).

The staining disappeared completely after fixation with formaldehyde (4%). After fixation with acetone the fluorescence staining pattern was the same as without fixation; however an enhancement of the intensity of staining of the cell walls was observed in the epidermis.

Blockade of the binding of antibodies to epithelial cell walls by Con A

In order to investigate the influence of pre-treatment with Con A on the interaction of pemphigus antibodies with epithelial cell boundaries the following experiments were done: unfixed cryostat sections of human oral mucosa and guinea-pig lip were treated with Con A (0·5 mg/ml) for 20 min, washed with PBS (3 × 5 min), incubated with a pemphigus serum* (diluted 1:50) for 30 min, washed with PBS, stained with HoAHuIg-FITC (1:60) for 30 min and washed with PBS.

In an analogous experiment sections were treated with Con A, incubated with rabbit anti-bovine oesophageal epithelium serum (1:10) and stained with HoARaIg-FITC (1:20).

Pre-treatment with Con A appeared to inhibit the interaction of pemphigus antibodies, as well as rabbit anti-epithelium serum with epithelial cell membranes of human oral mucosa and guinea-pig lip.

DISCUSSION

With Con A we were able to demonstrate specific glycoproteins in the cell surface coat of stratified squamous epithelium of human skin, human oral mucosa and guinea-pig lip. In this respect both the Con A-FITC method and the Con A-HRP method appeared to be very useful; moreover they gave a similar staining pattern. The same pemphigus-like staining pattern was observed by Nieland (1973) in the epithelium of human skin and monkey oesophagus, using fluorescent Con A- and phytohaemagglutinin P-conjugates. Inhibition and blocking tests showed the specificity of the staining with Con A. Furthermore periodate, which is known to oxidize 1,2 glycol groups in carbohydrates, was found to destroy the Con A receptor sites. Con A as well as pemphigus antibodies show reactivity with cell walls of stratified squamous epithelium. Ethanol and methanol treatment appeared to remove the Con A reactivity. The antigens reactive with pemphigus antibodies can also be removed by these fixatives (Beutner *et al.*, 1970). Acetone fixation can enhance staining reactions of pemphigus antibodies (Beutner *et al.*, 1970), as well as the staining with Con A.

The interaction of pemphigus antibodies with antigens on the epithelial cell membranes of human oral mucosa and guinea-pig lip could be blocked by pre-treatment with Con A. Rabbit anti-bovine oesophageal serum prepared by immunization of rabbits with an insoluble fraction of bovine oesophageal epithelium showed, in the indirect IF-test, a pemphigus-like staining pattern of squamous epithelia. This is in accordance with the findings of others (Ablin, 1969; Grob & Inderbitzin, 1967a; and Inderbitzin & Grob, 1967). The reaction of rabbit anti-bovine oesophageal antibodies with cell walls could also be blocked by Con A.

The results of our experiments indicate that the pemphigus antigens enclose the determinant groups as well as the Con A binding sites. Another possibility is that pemphigus antigens are not Con A-reactive and that the Con A-reactive sites are localized in the near vicinity of the pemphigus antigens.

The experiments of Grob & Inderbitzin (1967b) and also of Ablin *et al.* (1969) indicate that the pemphigus antigens are not identical with the human blood group substances A and B. Moreover, from the findings of Lloyd, Kabat & Beychok (1969) it is very unlikely that Con A reacts with blood group substances in human stratified squamous epithelium.

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*This serum was kindly supplied by Mr A.L.van Rossum of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (head: Professor Dr J.J.van Loghem). The IF-titre was 1:1000.

Note

During revision of this article Hashimoto, K. *et al.* (1974) published a paper, 'Identification of the substance binding pemphigus antibody and concanavalin A in the skin'. Their findings confirm our observations and conclusions.

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Antireticulin antibodies in dermatitis herpetiformis and adult coeliac disease

THEIR RELATIONSHIP TO A GLUTEN FREE DIET AND JEJUNAL HISTOLOGY

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SUMMARY

Antireticulin antibody was present in 17% of sera from thirty-five patients with dermatitis herpetiformis, 45% from twenty-eight patients with adult coeliac disease and none of thirty healthy controls.

There was a higher incidence of antireticulin antibody (a) in patients on normal diets than in those on gluten free diets, and (b) in patients with subtotal villous atrophy than in those with partial villous atrophy. Two patterns of tissue staining were demonstrated, one of which was almost invariably associated with jejunal subtotal villous atrophy. The Ig class of the antibody was IgG in all instances but in 64% antibody of the IgA class was also present.

Antireticulin antibodies (ARA) have been found in increased prevalence in both adult coeliac disease (ACD) (Seah *et al.*, 1971a; Alp & Wright, 1971; Seah *et al.*, 1973; Lancaster-Smith *et al.*, 1974), and dermatitis herpetiformis (DH) (Seah *et al.*, 1971a, 1973). The significance of ARA is not clear. However, because the greatest incidence of these antibodies in patients with ACD (Alp & Wright, 1971; Seah *et al.*, 1973; Lancaster-Smith *et al.*, 1974), and DH (Seah *et al.*, 1973) occurs in those not on gluten free diets, it has been suggested that there may be antigenic cross reactivity between gluten and reticulin (Seah *et al.*, 1973).

A further explanation for the occurrence of ARA in ACD and DH is that it is produced in response to reticulin contained in food which has passed across the damaged jejunal mucosa. This is thought to be the origin of antibodies to other food constituents found in these diseases (Kumar *et al.*, 1973). Another suggestion is that ARA is a response to non-specific damage of small bowel mucosa (Alp & Wright, 1971). If such proposals were true ARA would be expected to occur most frequently in patients with the most severe small bowel mucosal lesions and those who also have antibodies against other food constituents.

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In a previous communication we reported a correlation between ARA and antibodies against macromolecules of dietary origin in ACD and DH (Kumar *et al.*, 1973). Attempts have now been made in this study to relate the occurrence of ARA to jejunal mucosal abnormality and the presence or absence of gluten in the diet, in the hope of clarifying the relevance of this antibody.

PATIENTS AND METHODS

Thirty-eight sera from thirty-five patients with dermatitis herpetiformis (male 26, female 9; age range 17–73 years), and thirty-three sera from twenty-eight patients with adult coeliac disease (male 7, female 21; age range 19–71 years) were examined and compared with sera from thirty normal controls (male 14, female 16; age range 20–71 years).

Of the thirty-eight DH sera, thirty-two were obtained whilst the patients were on normal diets and six whilst on gluten free diets. Three patients were examined before and after starting a gluten free diet. Jejunal biopsies at the time of obtaining the sera showed a normal mucosa in five, partial villous atrophy in nineteen and subtotal villous atrophy in fourteen.

The thirty-three sera from ACD patients were taken from eleven patients on normal diets and from twenty-two on a gluten free diet. Five patients were examined before and after institution of a gluten free diet. The jejunal biopsies at the time of obtaining sera consisted of one with a normal mucosa, twelve with partial villous atrophy and twenty with subtotal villous atrophy.

Tissue auto-antibodies

A one-in-ten dilution of serum was examined by standard indirect immunofluorescent methods for the presence of antireticulin antibodies (Seah *et al.*, 1971a), using cryostat sections of rat liver, kidney and stomach as antigen. The results were read without knowledge of the diagnostic status of the patients. Polyvalent antihuman immunoglobulin fluorescein conjugate (Burroughs Wellcome) was used for the initial screening and positive sera were subsequently tested using specific antisera for IgA, IgG and IgM (Behringwerke), at dilutions chosen following block titration. Examination of the stained sections was made on a Reichert microscope incorporating an iodine quartz light source with Balzer F.I.T.C.3. and Kodak Wratten gelatin 12 filters.

RESULTS

Antireticulin antibody was increased in prevalence in both DH (17%) and ACD (45%), compared with the control group in which ARA was not found.

Relationship to a gluten free diet (See Table 1)

Dermatitis herpetiformis. Six (19%) of thirty-two patients whilst on normal diets and one (16%) of six on gluten free diets had ARA in their sera.

Adult coeliac disease. Nine (75%) of twelve patients on normal diets and six (29%) of twenty-one patients on gluten free diets had ARA in their sera. This difference is statistically significant ($P < 0{\cdot}05$).

Relationship to jejunal mucosal morphology (See Table 2)

Dermatitis herpetiformis. Of fourteen patients with subtotal villous atrophy six (44%) had ARA compared with only one (5%) of nineteen with partial villous atrophy and none of five with a normal mucosa. This difference between patients with subtotal villous atrophy and those with partial villous atrophy and normal mucosae is significant ($P < 0.01$).

TABLE 1. Relationship of antireticulin antibody to diet

Group	Normal diet		Gluten free diet	
	Total	ARA positive	Total	ARA positive
DH	32	6 (19%)	6	1 (16%)
ACD	12	9 (75%)	21	6 (29%)

DH, dermatitis herpetiformis; ACD, adult coeliac disease; ARA, antireticulin antibody.

TABLE 2. Relationship of antireticulin antibody to jejunal mucosal morphology

Group	Subtotal villous atrophy		Partial villous atrophy		Normal mucosa	
	Total	ARA positive	Total	ARA positive	Total	ARA positive
DH	14	6 (44%)	19	1 (5%)	5	0
ACD	20	11 (55%)	12	4 (33%)	1	0

DH, dermatitis herpetiformis; ACD, adult coeliac disease; ARA, antireticulin antibody.

TABLE 3. Relationship of antireticulin antibody staining pattern to diet and jejunal mucosal morphology

Group	Staining pattern	Total	Untreated	Treated	STVA	PVA	Normal mucosa
DH	Type 1	5	5	0	5	0	0
	Type 2	2	1	1	1	1	0
ACD	Type 1	6	4	2	5	1	0
	Type 2	9	5	4	6	3	0

DH, dermatitis herpetiformis; ACD, adult coeliac disease; STVA, subtotal villous atrophy; PVA, partial villous atrophy.

Adult coeliac disease. Of twenty patients with subtotal villous atrophy eleven (55%) had the antibody and of twelve with partial villous atrophy four (33%) had positive ARA tests. The one patient with a normal mucosa did not have the antibody. This difference between ACD patients with subtotal villous atrophy and those with partial villous atrophy does not reach statistical significance.

Of the thirty-four patients (20 ACD, 14 DH) with subtotal villous atrophy seventeen (50%) had ARA compared with only five (14%) of thirty-seven (13 ACD, 24 DH) who had less severe or no jejunal lesions. This difference is significant ($P < 0{\cdot}01$).

Relationship to pattern of staining

The staining observed was of two distinct patterns (see Table 3).

Type 1. The predominant pattern was of well defined staining of fine advential fibres of blood vessels and sinusoids in the liver, of the peritubular and pericapsular fibres in the kidney and of those fibres adjacent to the basement membrane of gastric glands.

Type 2. In the liver the predominant staining appeared to be in the cytoplasm of Kupffer cells but, in addition, there was frequently patchy staining of sinusoidal fibres. This was usually accompanied by staining of the connective tissue between kidney tubules and between the glands of the stomach mucosa, which contrasted with staining of only well defined fibres adjacent to basement membranes found in Type 1.

Dermatitis herpetiformis. Five of the seven positive sera gave the Type 1 pattern of staining and all five were from patients on normal diets who had subtotal villous atrophy of the jejunal mucosa. Two patients had Type 2 staining, one of whom was on a normal diet and had partial villous atrophy, and the other, although taking a gluten free diet, still had subtotal villous atrophy.

Three patients' sera before treatment gave a Type 1 pattern. After taking a gluten free diet for 4–7 months, two had negative antireticulin antibody tests, and in the third a Type 2 pattern was present. In no case did the reverse occur.

Adult coeliac disease. Six of the fifteen positive sera gave the Type 1 pattern, five of these were from patients with subtotal villous atrophy, of whom four were on normal diets. This same pattern was also found in two patients on gluten free diets, one with partial villous atrophy and the other with subtotal villous atrophy.

Of the nine cases with the Type 2 pattern, six had subtotal villous atrophy and three partial villous atrophy. Five of these nine were on normal diets and four on gluten free diets.

Five patients were studied before and after starting a gluten free diet. Two of three patients, whose sera originally gave a Type 1 staining pattern converted to a Type 2 distribution 12 months after starting a gluten free diet. The third case had no antibody after 3 months on a gluten free diet. Two patients whose sera before treatment gave the Type 2 staining had no antibody when retested between 5–7 months later.

Of the eight patients (five ACD and three DH) studied before and after a gluten free diet, only two (one ACD and one DH) failed to show an improvement in the mucosal lesion and these were two of the three patients who continued to be ARA positive.

Immunoglobulin Class of Antibody. In all twenty-two positive tests the antireticulin antibody was of the IgG class and in fourteen it was also of the IgA class. There was no correlation between the immunoglobulin class of the antibody and whether the patient had DH or ACD. Similarly, there was no correlation with the type of staining pattern, dietary status or the severity of the mucosal lesion.

DISCUSSION

Our results confirm the increased prevalence of antireticulin antibody in both DH (Seah *et al.*, 1971a, 1973) and ACD (Seah *et al.*, 1971a, 1973). The demonstration of ARA in 45% of our ACD patients and 17% of DH patients compares with other reported incidences ranging from 34–40% in ACD and 17–22% in DH. We have confirmed the relationship in ACD between the occurrence of ARA and the presence of gluten in the diet (Seah *et al.*, 1971a, 1973). Other studies have also found the same association in DH (Seah *et al.*, 1973). We, too, found a slightly higher incidence in DH patients on normal diets than in those on gluten free diets, but the treated group was too small to make a meaningful comparison. This correlation between antireticulin antibody and the presence of gluten in the diet has lead to the suggestion that there is direct cross-reactivity between reticulin and gluten (Seah *et al.*, 1973), which is supported by Alp & Wright's (1971) demonstration of a correlation between ARA and high titre serum antibodies to gluten. We, however, did not confirm this association (Kumar *et al.*, 1973). This difference may be due to the relative insensitivity of the precipitin test for gluten antibodies used by us, compared to the tanned red cell technique employed by Alp & Wright. This theory fails to explain why all untreated patients with gluten sensitive enteropathy do not have antireticulin antibody. It has, however, been proposed that in such subjects all of the antireticulin antibody is bound to tissues rather than being free in the serum (Seah *et al.*, 1973). An alternative explanation is that these patients with negative antireticulin antibody tests were taking insufficient amounts of gluten in the diet at the time of the study to stimulate antireticulin antibody production. Conversely, the most likely explanation for this antibody occurring in patients on gluten free diets would be failure to adhere to a strict dietary regime.

An alternative to the cross-reactivity theory is that ARA is produced in response to non-specific damage of small bowel mucosal reticulin, which might explain why ARA occurs not only in gluten sensitive enteropathies, but also in 25% of Crohn's disease patients. Thus, the highest incidence of antireticulin antibody in ACD and DH might be expected in those patients with the severest jejunal lesions. This proposal is supported by the demonstration in our study that ACD and DH patients with subtotal villous atrophy have a comparable incidence of ARA and that in both diseases the incidence was greater than in those with partial villous atrophy or normal mucosae. Furthermore, a possible explanation for the greater incidence of ARA in ACD patients with partial villous atrophy than in DH patients with partial villous atrophy is that in all of the ACD cases the jejunal lesion had previously been subtotal villous atrophy. This contrasts with the situation in DH where the majority had shown partial villous atrophy when first investigated. A further possible explanation for the lower prevalence in DH is the less extensive jejunal abnormality and in particular the possibility that the lesion has a patchy distribution in this condition. In addition, it should be noted that the low prevalence of ARA in DH patients with partial villous atrophy cannot be explained by absence of gluten in the diet as only three of these nineteen patients were on such treatment. It appears from our findings, therefore, that the present or recent past severity of the mucosal lesion *per se* is important in the pathogenesis of ARA, which contrasts with the conclusion of Seah and his group (Seah *et al.*, 1973).

It is clear, however, that the severity of the mucosal lesion is not the only factor in the production of ARA, for children with cow's milk intolerance (Von Essen, Savilahti & Pelkonen, 1972), and adults with tropical sprue (Seah *et al.*, 1973), who have similar small intestinal lesions to those of gluten sensitive enteropathy, do not have ARA. Furthermore, ARA occurs in IgA deficient subjects with normal jejunal mucosa (Von Essen *et al.*, 1972). It may be of importance that IgA deficiency is commonly associated with auto-immune phenomena (Hobbs, 1968) and it has been proposed that in coeliac disease there is a defect in the IgA system (Hobbs, 1971). It is tempting to propose that the gluten induced mucosal lesion and an IgA defect combine to predispose to the production of ARA in coeliac disease and dermatitis herpetiformis.

Alp & Wright (1971) have proposed that antibodies against reticulin may be a response to reticulin contained in food crossing the pathologically permeable jejunal mucosae of coeliac patients. We have previously reported a positive correlation between the presence of antireticulin antibodies and antibodies against a wide range of food constituents, which would tend to support this suggestion (Kumar *et al.*, 1973). However, in DH and ACD the prevalence and titre of antibodies against food constituents are greatest in those patients with subtotal villous atrophy (Kumar *et al.*, 1973). Thus, the high incidence of antireticulin antibodies in these same patients with subtotal villous atrophy may be due to the jejunal damage *per se* as discussed previously, rather than to passage of dietary reticulin across the mucosa.

Distinctive patterns of reticulin staining were noted by Seah *et al.* (1971) and Rizzetto & Doniach (1973) but they did not correlate these with dietary status or jejunal histology. Types 1 and 2 patterns in our study would appear to correspond closely to some of these previously described staining patterns. Of our eleven patients with the Type 1 staining all but one had subtotal villous atrophy and only two were on gluten free diets. The significance of these different patterns is not known. It is probable that there are at least two antibodies against distinct antigenic constituents of reticulin. However, it seems from our study of patients before and after treatment that both types of staining may disappear and that Type 1 converts to Type 2 in some instances, following withdrawal of gluten from the diet and improvement in the jejunal lesion.

We have confirmed that when ARA occurs it is invariably of the IgG class. This study also shows that in 64% of cases with IgG-ARA there is also an antibody of the IgA class, which compares very closely with the incidence in childhood coeliac disease (Seah *et al.*, 1971b).

We consider that screening for antireticulin antibody is a useful additional investigation to detect untreated patients with ACD, and DH patients with subtotal villous atrophy. Serial observations of patients before and after treatment indicate that patients with positive tests do become negative for ARA. Failure to do so was associated with continuing subtotal villous atrophy and this may well have been the result of not keeping to a gluten free regime. The test may, therefore, also be of use in monitoring adherence to a gluten free diet.

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Plasma β-MSH levels in acne vulgaris

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SUMMARY

Plasma β-MSH levels and sebum excretion rates (SER) were measured in thirty male and twelve female patients with acne vulgaris. The mean SER in both male and female patients was significantly increased as compared to normal control levels.

Plasma β-MSH levels were normal in male and female patients with acne and showed no correlation with the SER. The SER that occurs in acne cannot therefore be explained by an increased secretion of β-MSH.

Administration of α-MSH stimulates sebum secretion in the rat (Thody & Shuster, 1971a, 1973) and removal of the intermediate lobe, the site of MSH production, leads to a large decrease in sebum secretion that is restored by α-MSH (Thody & Shuster, 1972, 1973). We have therefore proposed that α-MSH or a related peptide has an important role as a sebotrophic hormone in the rat (Thody & Shuster, 1973; Shuster & Thody, 1974). A melanocyte-stimulating hormone may also be responsible for the seborrhoea that occurs in certain clinical conditions (Burton *et al.*, 1973). Although the identity of this sebotrophic hormone has not been established it has been assumed to be a β-MSH since this is thought to be the major MSH in man (Harris, 1959; Abe *et al.*, 1967), and the seborrhoea of Parkinsonism is associated with an increase in β-MSH secretion (Shuster *et al.*, 1973). Increased sebum production also occurs in acne and this has been related to the severity of the condition (Pochi & Strauss, 1964; Cunliffe & Shuster, 1969a; Burton & Shuster, 1971). We therefore wondered whether the seborrhoea of acne could be related to an increased secretion of β-MSH.

METHODS

Thirty male patients aged 14–24 years and twelve female patients aged 15–28 years with acne vulgaris were examined. The severity of the acne as assessed by Burton *et al.* (1971) ranged from Grades 1–4 in the males and 2–4 in the females.

The sebum excretion rate (SER) was measured from forehead skin by the method of Strauss & Pochi (1961) as modified by Cunliffe & Shuster (1969b). The plasma β-MSH was measured by a radio-immunoassay method (Thody & Plummer, 1973).

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All blood samples for β-MSH estimation were taken at 2 p.m. during the measurement of the SER. Because of the premenstrual worsening of acne, plasma β-MSH levels and SER were measured both 2–3 days before and 7–10 days after menstruation.

The SER was compared with the normal range obtained from thirty-four female and thirty-two male control patients aged 10–29 years. Plasma β-MSH levels were also measured in thirty-six male and female controls aged 16–56 years. This control group consisted of nineteen normal subjects attending as outpatients for the treatment of common skin disorders such as psoriasis and eczema.

RESULTS

The mean SER ± SEM in thirty-two male control subjects was 0·98 ± 0·09 μg/cm^2/min and 0·63 ± 0·06 μg/cm^2/min in thirty-four female control subjects. In thirty male subjects with acne vulgaris the mean SER ± SEM was 1·59 ± 0·10 μg/cm^2/min (range 1·02–2·74 μg/cm^2/min), and this was significantly different ($P<0\cdot01$) from the male control value (see Table 1). The mean SER ± SEM in twelve female patients 2–3 days prior to menstruation was 1·79 ± 0·21 μg/cm^2/min (range 1·08–2·93 μg/cm^2/min). This was significantly different ($P<0\cdot001$) from the female control value (Table 1).

TABLE 1. Sebum excretion rates in male and female patients with acne vulgaris. The figures in parentheses refer to the number of patients examined. The student's *t*-test was used to test for significant differences. All values are means ± s.e.mean.

	Sebum Excretion Rate μg/cm^2/min			
	Controls		Acne Vulgaris	*P*
Males	0·98 ± 0·09 (32)		1·59 ± 0·10 (30)	<0·01
Females	0·63 ± 0·06 (34)	pre-menstrual	1·79 ± 0·21 (12)	<0·001
		post-menstrual	1·56 ± 0·15 (10)	<0·001

Seven to ten days after menstruation the mean SER ± SEM obtained in ten of the female patients was 1·56 ± 0·15 μg/cm^2/min (range 0·90–2·10 μg/cm^2/min) and this was also significantly different ($P<0\cdot001$) from the control value (Table 1). Although the post-menstrual SER was lower than the pre-menstrual value the difference was not significant.

The mean plasma β-MSH level ± SEM in twenty-four male control subjects was 65·6 ± 6·9 pg/ml and in twelve female control subjects 59·9 ± 13·8 pg/ml. These values were not significantly different and the mean plasma β-MSH level ± SEM in all thirty-six control subjects was 63·7 ± 6·4 pg/ml. The mean plasma β-MSH level ± SEM in thirty male patients with acne vulgaris was 62·1 ± 7·6 pg/ml and this was not significantly different from the control value (Fig. 1). There was no correlation between the SER and the plasma β-MSH levels in these thirty male patients with acne (Fig. 2). The mean plasma β-MSH in eleven female patients 2–3 days prior to menstruation was 31·5 ± 3·4 pg/ml and in ten of those patients in whom it was also measured 7–10 days after menstruation, the mean plasma β-MSH ± SEM was 51·6 ± 10·4 pg/ml (Fig. 1). Whilst the post-menstrual level was

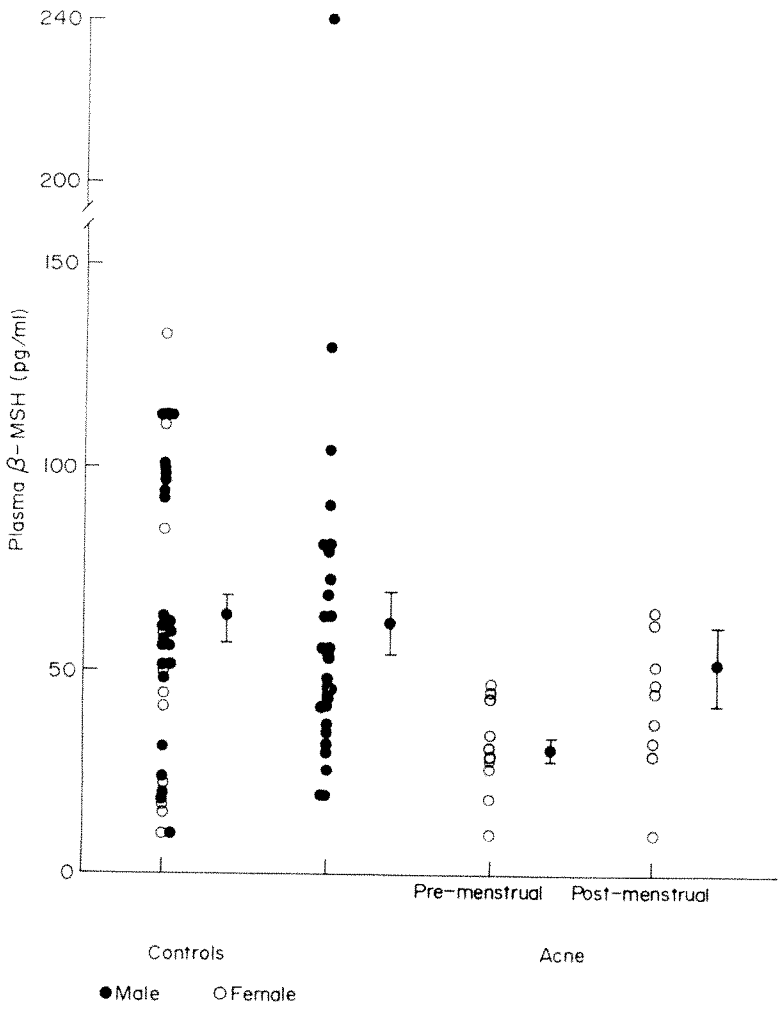

FIGURE 1. Plasma β-MSH levels in male and female patients with acne vulgaris. In female patients plasma samples were taken 2–3 days prior to and 7–10 days after menstruation. The mean ± s.e. mean for each group is indicated by the vertical line. There was no significant difference between the values obtained in male and female control subjects. The mean plasma β-MSH level in the male acne patients was not significantly different from the control value. There was no significant difference between the pre-menstrual and the post-menstrual plasma β-MSH levels in female acne patients, and the post-menstrual level was not significantly different from the control value. The pre-menstrual level was significantly lower ($P<0{\cdot}001$) than the control value but not significantly different from the level in the twelve female control subjects. Student's *t*-test was used to test for significant differences.

not significantly different from the control value the pre-menstrual level was significantly lower ($P<0{\cdot}001$). However, the pre-menstrual level was not significantly decreased when compared with the level in the twelve female control subjects. As in the males no correlation was found between the SER and the plasma β-MSH levels in female subjects with acne (Fig. 2).

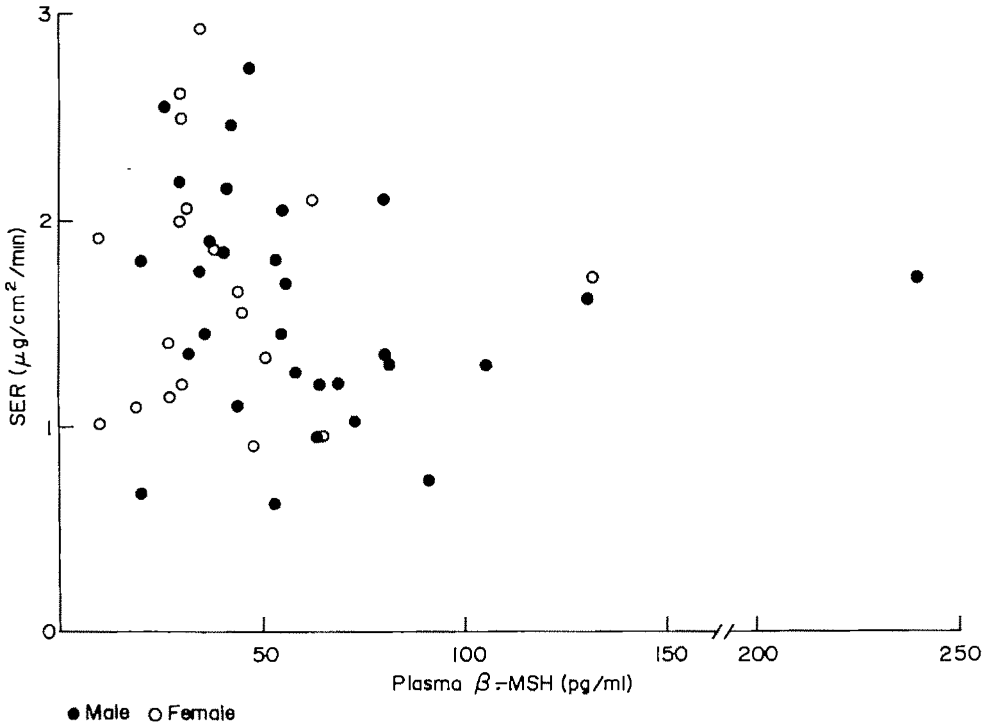

FIGURE 2. Plasma β-MSH values in male and female patients plotted against the SER to show lack of correlation.

DISCUSSION

An increased rate of sebum excretion is an essential feature of acne vulgaris (Pochi & Strauss, 1964; Cunliffe & Shuster, 1969b) and this was confirmed in the present study. The cause of this seborrhoea is unknown, but since the sebaceous glands are mainly under an endocrine control in man (Goolamali, Burton & Shuster, 1973; Goolamali *et al*, 1974), the seborrhoea may be related to an overproduction of a sebotrophic hormone, an enhanced response to such a hormone or both. The sebotrophic hormone was originally considered to be testosterone, but plasma levels and urinary excretion of testosterone are normal in acne vulgaris (Pochi *et al.*, 1965; Scroggins, Briefer & Kliman, 1965). The present results similarly demonstrate that plasma β-MSH levels are not abnormal in acne vulgaris and the seborrhoea of acne cannot therefore be explained by an increased production of this hormone. However, although β-MSH is thought to be the major melanocyte stimulating hormone in man the human pituitary does contain other forms of MSH (Abe *et al.*, 1967; Desranleau, Gilardeau & Chretien, 1972). Two such peptides, α-MSH and β-LPH, are known to be strongly sebotrophic in the rat (Thody & Shuster, 1971a, b; 1973) and secretion of these must now be examined in acne vulgaris.

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The erythemal action spectrum and deoxyribonucleic acid repair synthesis in xeroderma pigmentosum

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SUMMARY

The reactions to monochromatic radiation of the skin of ten patients with xeroderma pigmentosum were investigated, and eight were abnormal. The abnormalities consisted of papular and vesicular reactions and delay in the development of the minimal erythema dose reaction with wavelengths principally in the 290–320 nm range. Three patients were too young for full action spectra to be obtained but of those patients in whom all wavelengths were tested only one showed a reaction to radiation above 320 nm and this was at 340 nm only. In only one patient was the minimal erythema dose at 300 nm at 24 h lower than normal.

In six patients the repair synthesis of deoxyribonucleic acid after ultraviolet radiation was estimated. Reduced levels were seen in five but repair was normal in one patient. The patient with normal repair also had normal reactions to monochromatic radiation.

The abnormal reaction of the skin to artificial radiation and the abnormal deoxyribonucleic acid repair synthesis may enable the diagnosis of xeroderma pigmentosum to be made at a very early age. In one of the patients the diagnosis was made at the age of 6 months in a child with photosensitivity but with no other clinical signs of the disease. It is suggested that, by making the diagnosis as early as possible and by protecting the skin from natural sunlight, cutaneous malignancies may be prevented.

Xeroderma pigmentosum (XP) is a recessively inherited disease in which there is abnormal sensitivity to sunlight and a marked predisposition to cutaneous malignancies which often develop in childhood. It is clear that the reaction of the skin to artificial ultraviolet radiation (UVR) is frequently abnormal in XP (Zoon, 1936; Rottier, 1956), but the earlier studies of Rothman (1923), Martenstein (1924) and Lynch (1934) are difficult to evaluate. The first reported full monochromatic action spectrum for erythema in this disease seems to be that of Cripps, Ramsay & Ruch (1971).

The finding by Cleaver (1968) that fibroblasts grown from the skin of patients with XP failed to repair UVR induced damage to their deoxyribonucleic acid (DNA) was a major advance. Cleaver's results have since been confirmed by several workers (Setlow *et al.*, 1969; Bootsma *et al.*, 1970; Parrington, Delhanty & Baden, 1971; Giannelli, Croll & Lewin, 1973).

Reprint requests to Dr Ramsay.

We report here the results of irradiation of the skin with artificial UVR in ten patients with XP together with details of UVR induced DNA repair synthesis in fibroblasts of six of them.

MATERIALS AND METHODS

Skin tests with artificial UVR

Narrow waveband radiation was obtained by a water filled quartz prism monochromator and a 2 kW xenon arc source (Magnus *et al.*, 1959). The back of the trunk was irradiated with narrow wavebands at various doses throughout the UV and visible spectrum. The irradiated sites were examined at frequent intervals for the first 24 h, and thereafter daily wherever possible for 4–5 days. Particular note was made of the threshold dose for, and the morphology of, a reaction at each waveband at

TABLE 1. Details of patients together with results of monochromatic UVR skin tests and DNA repair synthesis in skin fibroblasts *in vitro*

Case No.	Sex	Age when tested	Onset of photosensitivity	Onset of pigmentation	Tests with monochromatic UVR	DNA repair synthesis
1. Y.W. P.6401	Female	4 years	7 months	3 years	Abnormal 300–340 nm	20–25% of normal
2. J.M. M.85097	Female	8 years	3 months	2 years	Abnormal 300 and 307·5 nm	25–30% of normal
3. W.L. P.31262	Male	6 months	2 months	Not yet present	Abnormal* 300 and 307·5 nm	18% of normal
4. K.B. P.15159	Female	14 months	2 months	2 years	Abnormal* 300 and 307·5 nm	6% of normal at age 3½ years
5. R.F. G.15078	Female	27 years	Early infancy	Not known	Abnormal 260–310 nm	Reduced (see text)
6. C.P. P.17807	Female	18 years	Early infancy	3 years	Normal	Normal
7. C.B. P.1698	Female	4 years	2 months	2 years	Abnormal* 300 and 307·5 nm	N.D.
8. R.R. G.12210	Female	13 years	Not known	Early childhood	Abnormal 290–310 nm	N.D.†
9. E.R. G.7680	Male	15 years	Early infancy	Early infancy	Abnormal 290–310 nm	N.D.†
10. D.E. G.14962	Female	18 years	4 years	5 years	Normal	N.D.†

* 'Sunburn' UVR (300–320 nm) only tested; † Seen before the test was described; N.D., Not done. Cases 8 and 9 are sister and brother.

each time interval after irradiation. In some infants and young children it was only possible to test two or three wavebands in the 'sunburn' UVR (300–320 nm).

UVR induced DNA repair synthesis

Skin cultures were set up from patients and normal volunteers by a modification of the technique of Hsu & Kellog (1960). On subcultivation the cells were seeded in petri dishes containing coverslips and Dulbecco minimal essential medium (MEM) with 10% fetal calf serum. One hour prior to UV irradiation 10 μCi/ml of ^{3}H-Thymidine (^{3}H-Tdr) with a specific activity of 25 Ci/mmol were added to the culture medium in order to label differentially cells undergoing replicative DNA synthesis. The medium was then discarded and the cells irradiated with a germicidal lamp emitting UVR predominantly at 254 nm. The dose of irradiation was varied between 1 and 15 mJ/cm^2 and the rate adjusted so that irradiation time did not exceed 40 s. The cells were then incubated in medium containing 10 μCi/ml of ^{3}H-Tdr (spec. act. 12·5 Ci/mmol) and hydroxyurea (2×10^{-3}M). After 2 h they were washed in balanced salt solution, fixed for 15 min in 3:1 methanol glacial acetic acid mixture, washed in methanol, dried and processed for autoradiography (Giannelli, 1974). DNA repair synthesis was measured by counting the autoradiographic silver grains over cells not undergoing replicative DNA synthesis and dose response curves were drawn. The values indicated in Table 1 are the means estimated from such curves and expressed as a percentage of the repair synthesis of normal control cells. It was not possible to obtain a quantitative value for Case 5 although repair synthesis was obviously reduced.

PATIENTS AND RESULTS

There were eight females and two males. The age of the males was 6 months and 15 years and that of the females ranged from 14 months to 27 years when investigations were performed. Clinical details are summarized in Table 1. It is seen that all patients except one (Case 8) experienced photosensitivity from an early age. The time of onset of this symptom in Case 8 was unknown. Pigmentation in the form of macules in the light exposed areas was present in all patients except the 6 month old child (Case 3) who is discussed in detail later. Four patients (Cases 4, 6, 7 and 8) have definitely had one or more tumours of the skin, basal cell epitheliomas, squamous cell epitheliomas or keratoacanthomas. With 3 patients (Cases 1–3) no tumours have yet developed but it should be stressed that these three patients are under the age of 9 years and may yet develop tumours. No information about the presence or absence of tumours is available in the remaining three patients (Cases 5, 8 and 9).

A definite history of consanguinity was obtained in only one patient (Case 6), no information was available in four (Cases 5, 8–10) and consanguinity was absent in the remaining patients. Two patients (Cases 8 and 9) were brother and sister but there was no family history of XP in the others.

Tests with artificial UVR

Table 1 shows that eight of the ten patients developed abnormal reactions. The results in those patients in whom skin irradiation tests with monochromatic UVR were abnormal are shown in Table 2. The dose for two different reactions is given at 24 h and 72 h after irradiation. The MED is the lowest dose in a graded series which produced erythema at the stated time and the minimum papular dose the lowest dose at which a papule developed. In four patients (Cases 2, 3, 5 and 7) the threshold for erythemal and for papular responses at 72 h was identical at one or more wavelengths. In normal subjects the MED at 300 nm when read at 24 h was within the range 9–120 mJ/cm^2 (Magnus, 1964) and in all patients but one (Case 4) the MED at 24 h was within this range. With Case 4 the MED was 5mJ/cm^2 which is not strikingly lower than normal.

TABLE 2. Doses required for minimal erythema and papular responses in patients with abnormal reactions to UVR. Doses are in mJ/cm^2

Case No.	Wavelength (nm)	MED	24 h Minimum papular dose	MED	72 h Minimum papular dose
1.	300	33	330	10	33
	307·5	400	1350	14	135
2.	300	97	320	11	97
	307·5	485	>485*	144	144
3.	300	10	50	10	10
	307·5	50	150	50	50
4.	300	5	50	5	50 Vesicle
	307·5	75	225	25	75 Vesicle
5.	300	26	86	9	9
	310	95	>2850*	95	95
7.	300	13	120	4	13
	307·5	54	>162*	54	54
8.	290	26	>230*	8	26
	300	115	>230*	38	115
9.	290	17	>150*	5	>150*
	310	33	100	33	100

* No papule developed.

Seven of the eight patients with abnormal reactions showed an MED at 72 h that was lower than that at 24 h; i.e. doses which at 24 h had produced no reaction did produce erythema at the later time. In addition the erythematous areas persisted longer than normal. An abnormal morphological response to UVR at one or more wavelengths at 24 h appeared in six patients. This reaction was in the form of a papule which developed after doses approximately three times the MED, a reaction not seen in normal persons. The exceptions were Cases 7 and 8. Case 7 did not develop a papule until a dose of approximately ten times the MED had been given at 300 nm and Case 8 did not develop a papular reaction at 24 h with the doses given. At 72 h after irradiation an abnormal papular response was present in all eight patients and in one (Case 4) a vesicle had appeared. One patient (Case 1) also developed a reaction at 340 nm, the appearance of which was delayed for 10 days.

Three patients (Cases 5, 8 and 9) were tested at 250–280 nm. Two patients (Cases 8 and 9) reacted normally, i.e. the MED reactions at 24 h first developed 6–8 h after irradiation and the reactions slowly faded over the next few days. The third patient (Case 5) showed a similar normal reaction at 250 and 280 nm but at 260 and 270 nm the MED at 48 h was lower than that at 24 h. No abnormal morphological reactions developed at these wavelengths.

Tests of UVR induced DNA repair synthesis

These are summarized in Table 1. In one patient (Case 7) this test has not yet been performed and three patients (Cases 8–10) were seen before this test was first reported. Five patients (Cases 1–5) in whom DNA repair has been investigated showed abnormal results with markedly reduced levels of repair when compared with normal controls. In another (Case 6), fibroblasts from two separate skin biopsies showed DNA repair activity which was within the normal range. The level in both specimens was slightly lower than the mean for normal controls but the difference was not significant.

DISCUSSION

Abnormal reactions after irradiation of the skin with broad spectrum UVR sources in patients with XP were first reported some years ago. Rothman (1923) showed, in one case, that regression of erythema was delayed and that telangiectasia developed at the peak of the erythema. A similar result was obtained by Martenstein (1924) again on a single case, and he showed that there was some difference between the reactions on clinically affected and on unaffected skin. The patient investigated by Lynch (1934) was a 10-year-old girl. Irradiation was with a high pressure mercury vapour lamp and filters and the results suggested sensitivity to wavelengths between 280 and 310 nm. Zoon (1936) investigated two brothers with XP and irradiated the skin of the trunk with a mercury lamp alone and with varying thicknesses of 0·25 mm glass filters which absorbed radiation below 280 nm. With the unfiltered lamp there was very little difference in the erythema between the patients and control subjects. However, when the glass filters were used the control subjects required twenty times more irradiation to produce a just visible erythema at 300 nm than did the oldest XP patient. The erythema with filtered UVR was delayed for 24 h in the patient whereas it appeared at 3 h in the control. More recently Rottier (1956) and Magnus (quoted by Moynahan, 1962) demonstrated delay in the appearance of erythema after monochromatic UVR in the 290–310 nm region.

The first detailed report of an action spectrum for erythema in a case of XP is that of Cripps *et al.* (1971). Reactivity in their case extended to 340 nm and at all wavelengths between 280 and 330 nm the appearance of erythema was delayed. There was a striking difference between the MED at 24 and 72 h. Action spectra studies which showed similar results on our Case 2 were previously reported by Kobza & Giannelli (1972).

Normal skin reacts to irradiation with UVR in the 'sunburn' part of the spectrum (290–320 nm) by the development of erythema. Moderate doses of this UVR near the MED give erythema which first appears approximately 6–8 h after irradiation and usually fades by about 36 h. Reactions from higher doses usually begin to develop earlier and to recede by 72 h after irradiation. Wavelengths above approximately 320 nm do not produce delayed erythema in normal skin.

The results in the majority of our ten patients are in contrast with the normal response to 'sunburn' UVR described above. The main abnormalities were a delay in the development of the MED reaction and an abnormal morphological response to irradiation. Thus seven of the eight patients showed an MED at 72 h which was lower than that at 24 h. Abnormal morphological reactions developed in six of the eight patients at 24 h and in all patients at 72 h at one or more wavelengths. Only one patient showed an MED at 24 h which was below normal and that was not markedly reduced.

Tests were also performed at 250 nm in three patients (Cases 5, 8 and 9) and the reaction was normal, i.e. the MED reaction at 24 h first developed 6–8 h after irradiation. Although this wavelength is not present in terrestrial sunlight it is very effective at producing erythema. A similar normal result at 250 nm was also reported by Rottier (1956) and Cripps *et al.* (1971). Two of our patients (Cases 8 and 9) also showed normal reactions at 260–280 nm but the third (Case 5) showed a delay in the development of the MED reaction at 260 and 270 nm with normal reactions at 280 nm. The significance of the abnormal reactions at 260 and 270 nm in this one patient is unknown. It is of interest that Cripps *et al.* (1971) found a similar delay in the development of the MED reaction at 280 nm in their patient but tests were not carried out at 260 and 270 nm. Investigation of other patients will be necessary before it is known whether abnormalities at these shorter wavelengths are a regular occurrence in XP.

The reaction to narrow waveband artificial UVR is not always abnormal in XP. Thus in two of the present patients (Cases 6 and 10) who had no distinguishing clinical features the action spectrum was normal. Erythema from 'sunburn' UVR developed and faded in the normal manner and no abnormal morphological reactions developed. However it would appear from our results that the reaction to 'sunburn' UVR is abnormal in the majority of patients with XP who may thus show one or more of the following: (1) The latent period between irradiation and the appearance of the MED reaction may be prolonged; (2) The morphology of the reaction may be abnormal; (3) The MED may be lower than in normal controls. These abnormal reactions may enable the diagnosis to be strongly suspected at an early age before any of the characteristic skin changes of XP develop. Thus in our Case 4 no cause was apparent for the photosensitivity which started at the age of 2 months. Tests with monochromatic UVR were performed at the age of 14 months when the skin was clinically normal. The delayed reaction described above developed but it was not until 9 months later that the typical freckling of XP first appeared. Skin fibroblasts cultured *in vitro* at the age of 3½ years showed only 6% of the normal DNA repair synthesis.

One of the most important changes which occurs in UV irradiated DNA is the formation of pyrimidine dimers (Setlow & Setlow, 1962; Setlow, 1966). Normal cells have a mechanism for the removal of these dimers and the insertion of new bases into the damaged DNA strand—so called excision repair. Cleaver (1968) was the first to demonstrate that fibroblasts cultured from the skin of some patients with XP were defective in this form of repair. Since then it has also been shown that epidermal cells and lymphocytes of several patients with XP are deficient in excision repair (Epstein *et al.*, 1970; Jung, 1971; Burk *et al.*, 1971). Bootsma *et al.* (1970) have shown that different patients with XP have different levels of excision repair and they suggested that the level is genetically determined and correlated with the severity of the disease. However, Cleaver (1972) and Robbins, Levis & Miller (1972) have recently described patients with clinically classical XP in whom excision repair is normal. A similar result was obtained in our Case 6.

The relationship between the abnormality in excision repair and the development of cutaneous malignancies in XP is not completely understood. The fact that patients with XP can have normal levels of repair suggests that a deficiency in the excision of pyrimidine dimers is perhaps a sufficient but not a necessary cause of UVR induced neoplasms. There may be a correlation between the defect in DNA repair and the abnormal reaction of the skin to artificial UVR (see Table 1). If this correlation is true it cannot yet be explained in molecular terms.

Xeroderma pigmentosum is a serious disease because of the associated cutaneous malignancies. It is possible that by protecting the skin against sunlight from an early age this complication may be avoided. Early diagnosis is therefore essential and the disease must be suspected in any infant or child with abnormal reactions to sunlight. Congenital porphyria (Gunther's Disease) and erythropoietic protoporphyria which may also present with photosensitivity at an early age can be excluded by appropriate quantitative porphyrin estimations.

The best way of establishing the diagnosis of XP before the characteristic skin changes occur is thus by testing the skin with monochromatic UVR and by estimating the DNA excision repair level in skin fibroblasts. The youngest patient in whom we have made the diagnosis of XP is a child of 6 months (Case 3) who first developed photosensitivity at the age of 2 months. Quantitative red cell porphyrins were normal. At the time of investigation the skin was completely normal but skin tests with artificial UVR were grossly abnormal. At 72 h after irradiation doses of UVR which at 24 h had produced only erythema gave rise to papular reactions and higher doses produced superficial necrosis of the skin or vesiculation. Skin fibroblasts cultured *in vitro* showed a level of DNA repair synthesis which was only 18% of a normal control. Strict protection against sunlight with clothing and sunscreening agents which protect against 'sunburn' UVR has been instituted and it is hoped that in this way cutaneous malignancies may be prevented.

Furthermore it should be stressed that early diagnosis in XP is important not only for the clinical management of the patients but also for the timely detection of parents at a high risk (1:4) of producing further affected children. The latter can be now diagnosed pre-natally by testing the DNA excision repair ability of amniotic cells cultured *in vitro* (Regan *et al.*, 1971). This may enable parents and doctor to choose between termination of pregnancy or post-natal protective therapy. Finally the prospects for the genetic prevention of XP have been further improved by the establishment of a technique for the detection of the carrier state of the disease (Giannelli & Pawsey, 1974). Unfortunately the rare form of XP with normal tests with monochromatic UVR and normal DNA repair synthesis will continue to escape pre-natal or early post-natal diagnosis and carrier detection tests.

Addendum

Regan *et al.* (1971) suggested a technique for the prenatal diagnosis of XP but they had no opportunity of testing their method of amniotic cells from an XP pregnancy. We have recently reported the first successful case of the prenatal diagnosis of XP in the second pregnancy of the mother of Case 3 in this present paper (Ramsay, C.A., Coltart, T.M., Blunt, S., Pawsey, S.A., Gianelli, F. (1974) *Lancet.* **ii**, 1109.

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The influence of treatment of the paralesional skin with dithranol on healing in psoriasis

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SUMMARY

Application of dithranol confined to the normal skin surrounding thirty-six psoriatic lesions produced improvement in twenty-four lesions, which was significantly more than the improvement noted in four of thirty-six control lesions left untreated. Such healing was temporary and slower than in lesions treated conventionally. It is suggested that the apparently normal skin around a psoriatic lesion under treatment influences the psoriasis.

The clinically unaffected paralesional skin in psoriasis has been shown to be histologically and histochemically abnormal. The epidermal metabolic activity is increased as demonstrated by increased activity of the enzymes of the energy producing pathways (Braun-Falco, 1963; Gordon & Johnson, 1967). In this area of the skin, Braun-Falco (1971) has described histochemical changes characteristic of parakeratosis in the epidermis and an increased number of mast cells and macrophages in the dermis. Histologically the spread as well as the healing of a psoriatic lesion is gradual, involving both epidermis and dermis.

It has been shown that both the uninvolved skin and the active lesion in psoriasis contain a factor in the dermis which stimulates the development of a new lesion. The serum from a patient recovering from psoriasis was shown to have an inhibitory effect on the Köbner reaction and a healing action on an active lesion (Stankler, 1969). It seems feasible that, apart from this systemic influence, a local factor or factors, formed just beyond the edge of a treated lesion, may spread inwards to induce healing in the lesion itself. Regression of a psoriatic lesion promoted by treatment of the paralesional skin, and excluding the lesion itself, would provide evidence for the existence of such factors. This study reports the results of paralesional treatment with dithranol in thirty patients with psoriasis.

MATERIALS AND METHODS

The problem of accurate application of dithranol to the skin was overcome by using a solution of

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TABLE 1. Results of paralesional treatment with dithranol in acetone compared with no treatment

Case No.	Age	Sex	Site	Size (approx) diam. (cm)	Duration	Degree of improvement	
						Treated	Control
1	65	F	Lumbar region	5	Chronic	+ +	+
			Buttock	4	Acute	+ +	−
			Buttock	4	Acute	+ +	−
2	14	M	Knee	5	Chronic	+ +	+
3	28	F	Knee	6	Chronic	+	−
4	14	M	Knee	5	Chronic	+	−
5	52	M	Upper arm	4	Acute	+ +	+
			Upper arm	3	Acute	+ +	−
6	64	F	Knee	6	Chronic	−	−
7	36	F	Forearm	4	Acute	−	−
8	34	M	Upper arm	4	Acute	+ +	−
9	60	M	Upper arm	6	Chronic	+	−
10	40	F	Knee	6	Chronic	−	−
11	36	F	Upper arm	4	Acute	−	−
12	16	M	Knee	5	Chronic	−	−
13	37	M	Thigh	4	Acute	+ +	−
14	26	F	Knee	5	Chronic	−	−
15	55	M	Knee	6	Chronic	−	−
16	30	M	Knee	4	Chronic	−	−
17	45	M	Thigh	4	Acute	+ +	−
18	30	F	Upper arm	3	Acute	−	−
			Upper arm	4	Acute	−	−
19	30	M	Forearm	4	Acute	+ +	−
20	51	M	Dorsum foot	8	Chronic	−	−
21	31	M	Forearm	2	Acute	+ +	−
				1·5	Acute	+	−
22	39	F	Elbow	2	Acute	+ +	−
				1·5	Acute	+ +	−
23	32	F	Thigh	3	Acute	+ +	+ +
24	28	M	Shoulder	1	Acute	+	−
25	66	M	Thigh	1·5	Acute	+	−
				1·5	Acute	+	−
26	45	F	Shin	1	Acute	−	−
27	27	F	Shoulder	2	Acute	+	−
28	23	F	Thigh	1·5	Acute	+ +	−
				1	Acute	+ +	−

	No. Lesions	No. Improved
Treated	36	24
controls	36	4

$P < 0{\cdot}001 \quad \chi^2 = 12{\cdot}89$

(−), No change; (+), no scaling; (+ +), no scaling, flattening and reduction in erythema.

dithranol in acetone applied with a small brush. The solution was quick drying (within a few seconds) and so excluded accidental spread of dithranol to the affected areas; it produced orange fluorescence under Wood's light (confirming the site of application) and eventually pigmentation of the treated skin. Dithranol in acetone solution was stored under refrigeration at 10°C and rendered stable by addition of 0·2% ascorbic acid.

Selected lesions with untreated control sites were studied in thirty in-patients receiving conventional therapy with dithranol in Lassar's paste to the remaining areas. In twenty-eight of these patients, one or more selected lesions were circumferentially painted, to a radius of approximately 1·5 cm beyond the edge, with dithranol in acetone solution and a similar lesion was left untreated. The solution was applied once daily and the concentration of the dithranol was increased from 0·1% to 0·5% until the site became slightly irritated. In the remaining two patients, very large plaques of psoriasis were selected for study. The dithranol solution was applied similarly but only to a part of the circumference of the lesion. In a second investigation one patient took part. Sixteen lesions on the right upper limb were treated circumferentially with dithranol in acetone paint, whilst the same number of lesions on the left upper limb were treated with the paint alone in a similar fashion. The accuracy of the application of dithranol was checked periodically by examination under Wood's light. The test areas were photographed before and at the end of treatment which ranged from 2 to 4 weeks. In all patients response to treatment was also assessed by reliable independent observers with no knowledge of the investigation. The amount of improvement was graded as follows: marked improvement—no scaling, flattening of the lesion, reduction in erythema (+ +), moderate improvement—no scaling (+), no change (−).

RESULTS

Lesions of less than 3 months duration were classified as acute, those of more than 3 months duration as chronic. The results of paralesional treatment are shown in Table 1.

There was significant improvement in twenty-four of thirty-six lesions treated paralesionally compared with only four of thirty-six lesions left untreated ($P<0{\cdot}001$, $\chi^2 = 12{\cdot}89$). Compared with no improvement in sixteen lesions treated paralesionally with the vehicle alone, there was significant improvement in fourteen of sixteen lesions treated paralesionally with dithranol ($P<0{\cdot}001$, $\chi^2 = 12{\cdot}07$). The results are shown in Table 2.

TABLE 2. Results of paralesional treatment with dithranol in acetone compared with treatment with acetone alone

Paralesional therapy	No. of lesions	Improved
Dithranol and acetone	16	14
Acetone	16	0

$P<0{\cdot}001$ $\chi^2 = 12{\cdot}07$.

In all the patients the improvement noted with paralesional treatment was slower than in the lesions treated conventionally. In Case No. 17, islands of normal skin appeared in the plaque. Fig. 1 illustrates the change produced in a chronic lesion by paralesional treatment. In all the patients, where healing was induced, initially there was a loss of scaling followed by a reduction in erythema over the entire

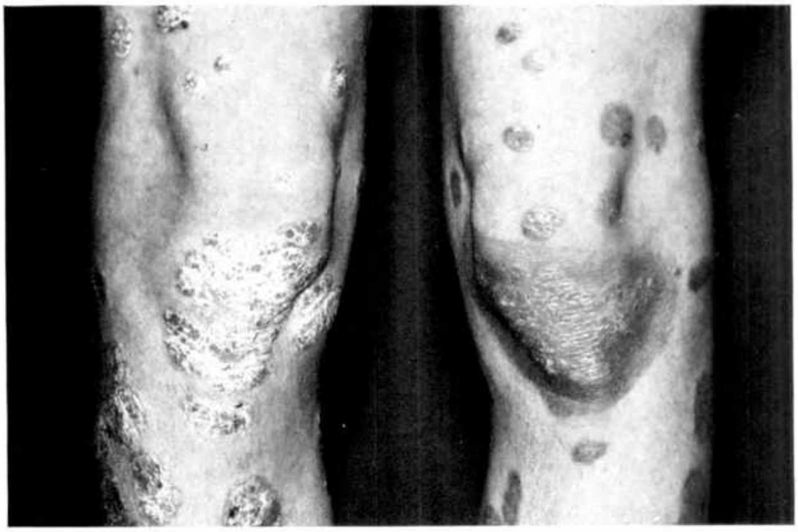

FIGURE 1. Paralesional treatment to plaque on left knee. Conventional treatment to other lesions left leg. Right leg not treated.

lesion. Psoriasis recurred at these sites, sometimes during the course of treatment. There was no relationship between the degree of paralesional pigmentation produced by the application of dithranol and the beneficial influence on the lesion.

DISCUSSION

Healing in the clinically affected skin, as compared with the control sites, could be induced in lesion, by treating exclusively the surrounding skin with dithranol. The response to such treatment was variable for unknown reasons and did not appear to be related to the duration, size or site of the lesion, the duration of therapy, or the concentration of the drug within the range tested. It is of interest that when healing was induced in the clinically affected skin, it did not progress centripetally, but affected the whole lesion. The regression was incomplete and temporary. It seems unlikely that the improvement, especially in the larger plaques, resulted from the diffusion of dithranol throughout the lesion. The loss of scaling followed by flattening of the lesions and a reduction in the erythema would, at present, suggest that the changes of resolution in the epidermis preceded those in the dermis.

The clinically unaffected skin in the immediate vicinity of an extending psoriatic lesion is the obvious site of a psoriagenic stimulus, whatever its nature. At the leading edge of the lesion the stimulus is manifested at its maximum, as suggested by histogeometric measurements of tissue changes in incipient and evolving lesions (Solani & Van Scott, 1972). The paralesional skin appears to be of some importance in a healing lesion as well. Theune & Wohlrab (1966) noted that after treatment with dithranol and ultraviolet light the earliest restoration of normal acid phosphomono-esterase activity was seen in the stratum corneum of the clinically healthy surrounding skin. In the conventional topical therapy of psoriasis, the paralesional skin is almost inevitably treated. The present study provides some evidence to suggest that, in healing in psoriasis, apart from systemic factors in the blood (Stankler, 1969), a local factor (or factors) formed in the clinically normal skin at the edge of a lesion may spread through the lesion and influence its regression.

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Microvascular response to local application of dequalinium chloride

A VITAL MICROSCOPICAL STUDY OF HAMSTER'S CHEEK POUCH AND A MICROANGIOGRAPHIC STUDY OF RABBIT'S EAR

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SUMMARY

Dequalinium chloride is a topical antiseptic, whose use may be attended by skin necrosis. Since microcirculatory changes are an early and distinct manifestation of tissue injury, the tissue damaging effect of dequalinium chloride on the microcirculation was examined.

The immediate vascular reactions were studied by vital microscopy of the hamster cheek pouch and the late effects were visualized by microangiography of the rabbit ear. An aqueous solution of dequalinium chloride in concentrations well below those used in clinical practice induced severe microcirculatory disturbances.

Dequalinium (1,1'-decamethylene bis[4-aminoquinaldinium chloride] is a cationic quaternary ammonium compound with a broad anti-microbial spectrum *in vitro* (Babbs *et al.*, 1965). In 1958 topical application of a 0·4% dequalinium cream or 0·5% paint was found to be of considerable value in pyococcal and candida infections of the skin, and appeared to be of value in tinea infections (Coles *et al.*, 1958). It was regarded as non-irritant (Coles *et al.*, 1958), and the side effects were some cases of dermatitis from dequalinium hypersensitivity (Coles *et al.*, 1958; Salo, Pirilä & Viljanen, 1968). Dequalinium became widely used against superficial infections of the skin and mucous membranes.

However, in 1965 necrotic ulcerations in the genital and perineal regions and body folds were attributed to the use of dequalinium for balanitis, vulvitis, napkin rashes and intertrigo (Coles & Wilkinson, 1965; Tilsley & Wilkinson, 1965). These complications were regarded as rare in relation to the widespread use of the antiseptic (Tilsley & Wilkinson, 1965; Putkonen & Salo, 1971). The agent was withdrawn in England which resulted in the disappearance of these remarkable cases of necrosis (Wilkinson, 1970). It is, however, still marketed in other countries for topical use against

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impetigo, infected dermatoses and wounds. It is obtainable without prescription, which means that the users are not advised to use it with care on lesions located in skin folds and the genital areas. Consequently, cases of skin and mucosal necrosis from dequalinium are still reported from Western Europe (Putkonen & Salo, 1971; Braun-Falco & Lukaes, 1970, Nörgaard, 1972, Wereide, 1972). These lesions have been located in the areas of predilection previously stated.

Very little seems to be known about the compound's mechanism of action on tissues. Since tissue injury is early and distinctly manifested by microcirculatory disturbances, the present study was performed to elucidate its influence on microvascular structure and function.

MATERIALS AND METHODS

The studies were performed on the hamster cheek pouch (thirty experiments) and the rabbit ear (sixteen experiments). Dequalinium chloride (kindly supplied by Glaxo Läkemedel AB, Mölndal, Sweden) was tested in the following concentrations: 1 mg/ml, 100 μg/ml and 25 μg/ml (corresponding to dilutions of 1:1000, 1:10,000 and 1:40,000). Distilled water was chosen as solvent, because dequalinium tended to precipitate in isotonic saline. Control experiments were done with distilled water. All solutions were sterile. The solutions were coded, and their contents were unknown to the investigators until all experiments were finished.

Short term experiments

Hamsters of both sexes, weighing about 100 g, were anaesthetized with Nembutal (30 mg/kg body weight) intramuscularly. The animal was immobilized on a special microscopic stage and the cheek pouch was everted and exposed over a thin glass plate so that a proper condensor system could be used for transillumination (Fig. 1). To maintain standardized local environmental conditions the

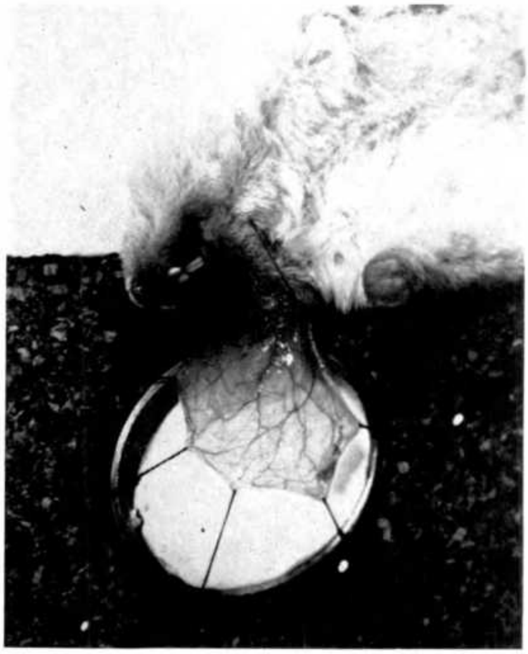

FIGURE 1. Bird's eye view of exposed cheek pouch of hamster.

cheek pouch was continuously irrigated with isotonic saline at a constant temperature, i.e. room temperature (20–22°C). The irrigation with saline is important as the circulation through the pouch is sensitive to dehydration. Some hamsters were rejected before the start of the experiment because of disturbed microvascular function after exposure of the pouch. Microvascular anatomy and pathophysiology were evaluated by vital microscopy. The studies were performed with a modified Leitz intravital microscope with transillumination (Brånemark & Jonsson, 1963; Brånemark, 1966). The microcirculation outside the experimental area was used as a continuous control.

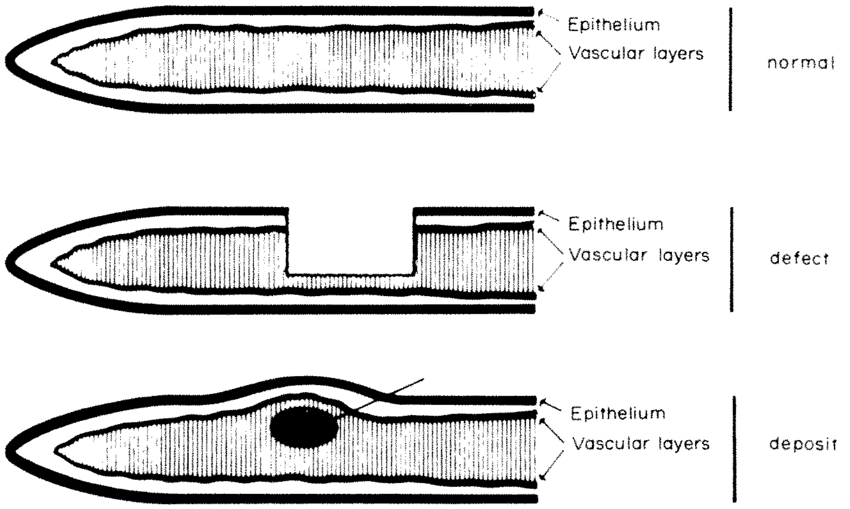

FIGURE 2. Diagrammatic representation of various experimental procedures. The normal exposed cheek pouch, small tissue defect and site of application into the tissue.

Two experimental designs were used: 1. The dequalinium or control solution was injected with a fine hypodermic needle (approx. volume 0·02 ml) into the tissue between the two vascular layers (deposit, Fig. 2). The circulation through the pouch was followed for up to 5 h. 2. Using a stereomicroscope and microsurgical instruments the epithelium and connective tissue on one side of the everted pouch were removed. This procedure resulted in a superficial wound approximately 2 mm in diameter with exposed connective tissue capillaries, arterioles and venules (defect, Fig. 2). The solution was applied drop-wise onto the wound and was removed after 15 min by irrigation with saline. The observations continued for 3–4 h.

Each pouch was as a rule used for three different deposits or defects, approximately 1 cm apart.

Changes in microvascular permeability were visualized by intravenous injection of Evans blue (vital dye T-1824, Mol. wt 960.83) in a dose of 15 ml/kg body weight. The dye becomes attached to circulating plasma albumin and forms a stable dye-protein complex which normally stays within the vessels. If the microvascular permeability is disturbed the dye-protein complex will leak and stain the tissue around the vessels (Rawson, 1943, Sörensen, 1972).

In defects, the structure of mast cells was delineated by *in vivo* staining with toluidine blue, 0·1% in saline.

Long term experiments

The solutions were injected (approx. 0·1 ml) subperichondrally on the outer side of rabbit's ear. On each ear two injections were given, one near the apex and the other towards the base. Each concentration of dequalinium was tested in both positions but in separate ears. The microvascular architecture and vascular proliferation at the site of the injection were revealed by microangiography on the 8th day with a standard technique regularly used at the laboratory (Lundskog, Brånemark & Lindström, 1968).

RESULTS

Short term experiments

Before any solution was administered the flow pattern was followed for 15 min to ensure that the experimental conditions were stable.

Deposits

Distilled water (seven experiments). Haemolysis occurred almost immediately. Within 2 min the circulation had stopped in almost all small (diameter less than 30 μ) and medium-sized (diameter 30–80 μ) vessels. The circulation soon recovered and after 15–30 min the flow was reconstituted in most vessels. However, signs of damage to the vessels could be seen with a few white thrombi and some granulocytes rolling along the vascular wall. Occasionally spherocytes could be seen. After 60 min the microvascular flow had recovered all over the deposit area (cf Brånemark *et al.*, 1967).

Slightly disturbed vascular permeability was visualized by a very slight blue-staining from Evans blue in the middle of the deposit.

Dequalinium chloride 1:40,000 (*seven experiments*). Within 30 s after the injection, haemolysis could be seen. In capillaries, small venules and arterioles there was complete stoppage of flow. In venules larger than 80 μ in diameter an immediate slowing of corpuscular flow velocity occurred. After a few minutes there could be seen in the border between the deposit area and surrounding tissues a moderate number of white emboli and granulocytes sticking to and sliding along the endothelium. Some spherocytes were also seen. Occasional segmental vascular contractions occurred. Swollen mast cells were not seen. Recirculation in capillaries and small venules and arterioles started after 20–40 min and the flow velocities gradually increased. Increased vascular permeability, as visualized by a slight leakage of Evans blue dye-complex, occurred in the periphery of the deposit area.

Dequalinium chloride 1:10,000 (*six experiments*). The immediate reaction was like the one with the lower concentration. However, after some minutes many vascular contractions were seen with markedly decreased flow. The circulation started again as with the lower concentration, but white thrombi more frequently adhered to the venular walls and constituted a hindrance to flow. The recirculation in capillaries and small venules and arterioles in the central part of the deposit was not as complete as with the lower concentration. The vascular contractions sometimes lasted for 3 h.

The longer the observation time, the clearer were the differences between the effect of this dequalinium concentration and that of the lowest.

Increased vascular permeability was visualized with Evans blue, and the staining of the tissue was more intense than the experiments with dequalinium 1:40,000.

Dequalinium chloride 1:1000 (*six experiments*). Within 30 s after the injection there was haemolysis and a complete stop of flow through the capillaries, small arterioles and venules. In medium-sized and larger vessels white thrombi and granulocytes could be seen sticking and sliding along the vessel walls (Fig. 3). Vascular contractions of larger vessels were a regular phenomenon starting within a few minutes. During the following 30 min more and more contractions were seen. The contractions were sometimes strong enough to occlude the vessel.

After 20–30 min mast cells were seen, mainly along large venules, because of their now optically dense granules (Fig. 3).

Hardly any vessels were seen showing recirculation. On the contrary, more and more vessels became occluded and within 120 min after the injection there was circulation only in the largest ones. This microcirculatory picture was unchanged during the following 3 h of observation.

The increased vascular permeability was visualized by Evans blue-complex leaking heavily in the periphery of the deposit, where circulation persisted.

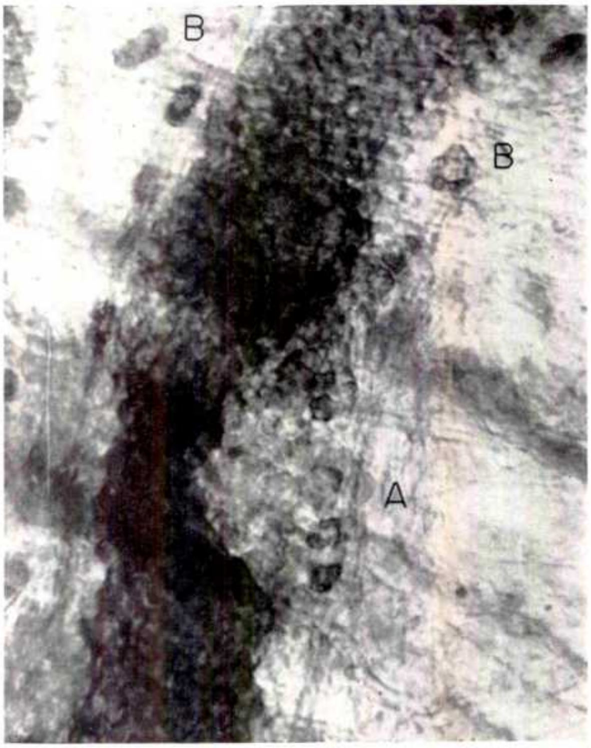

FIGURE 3. Formation of white thrombus (A) following application of dequalinium chloride, 1 : 1 000, to hamster cheek pouch. Some mast cells are seen (B) (× 300).

Defects

The preparation was intended to provide a wound model, a kind of tissue traumatization, representing one of the clinical conditions in which dequalinium may be used. However, in order to function as a test model the tissue has to be only moderately damaged. After a careful microsurgical preparation the microcirculatory system is functioning but is slightly disturbed. Such minimum tissue injury was manifested by one or two occluded venules, scattered granulocytes rolling along the endothelial wall and a few white thrombi in the test area.

Before the test solution was applied the flow pattern was noted and the pouch was irrigated with isotonic saline for 10–15 min which was found sufficient to obtain stable local conditions.

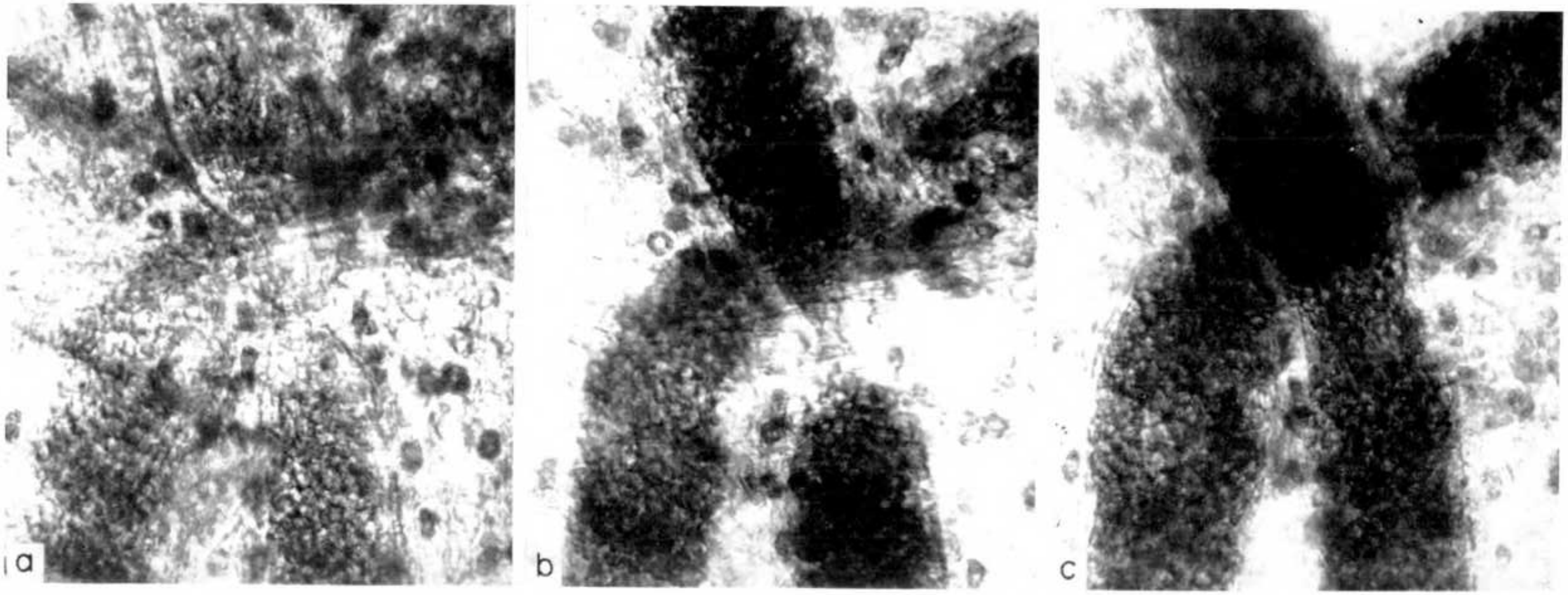

FIGURE 4a–c. In hamster cheek pouch defect two crossing venules are seen. In (a) one venule is contracted due to exposure for 15 min to dequalinium chloride, 1:1 000. (b) The dequalinium is removed and the contraction is not as prominent. A lumen can now be seen and blood flow is partly restored. (c) The contraction has almost relaxed (× 225).

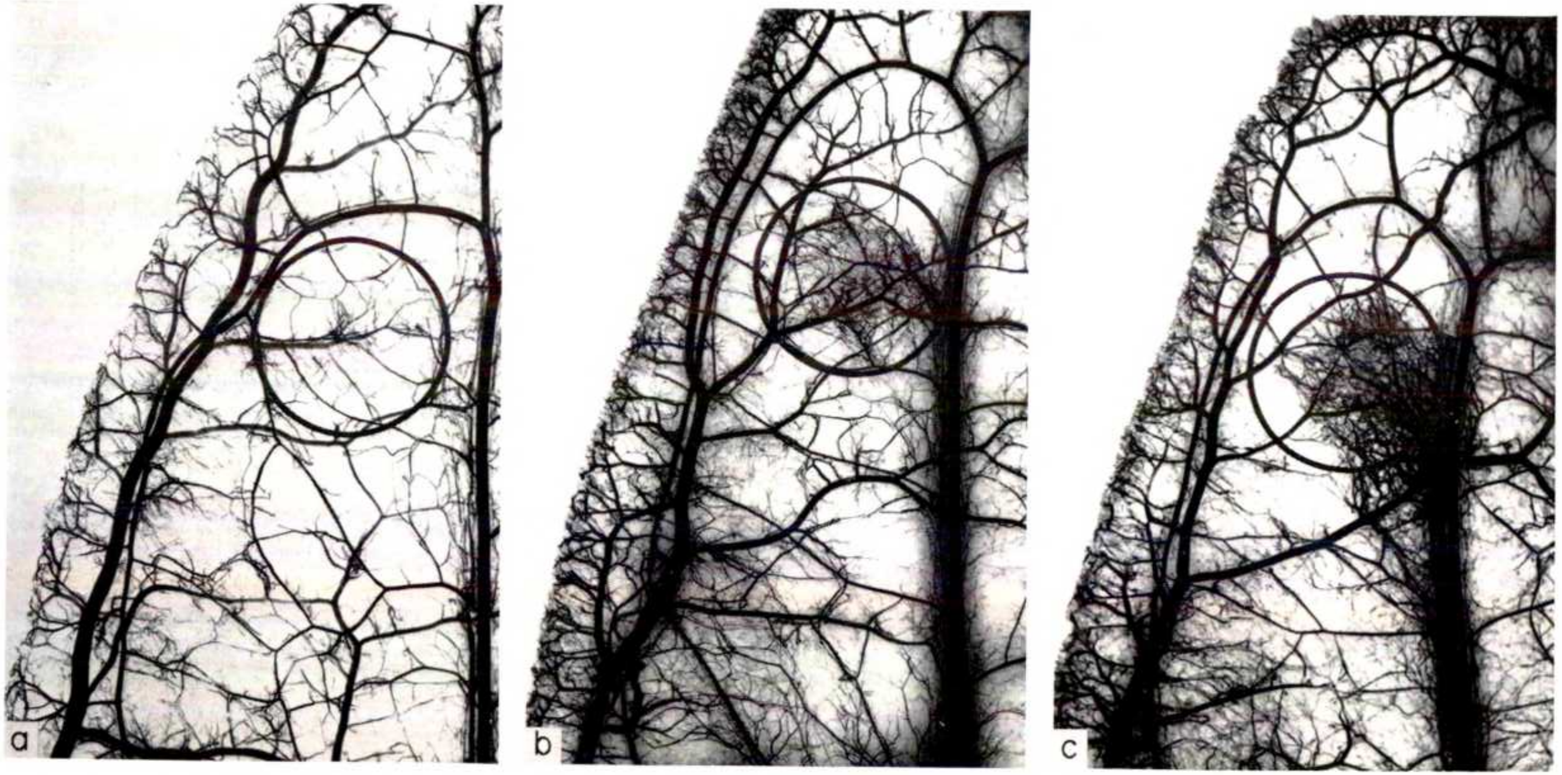

FIGURE 5a–c. Microangiograms showing microvascular system in rabbit's ear on day 8 after injection of 0.1 ml of the test substance. (a) A vascular reaction, very mild, to the solvent (distilled water). (b) Reaction to dequalinium chloride, 1:40,000, and here newly formed vessels can be seen. (c) Reaction to dequalinium chloride, 1:1000. Many newly formed vessels and some tortuous venules can be seen. The vascular reaction seen in (b) and (c) is typical of the second stage of an inflammatory reaction (× 2.5).

Dequalinium chloride in a concentration of 1 mg/ml (1:1000) was tested in four experiments. The immediate reaction after applying the drug was like that in the deposit experiments, i.e. haemolysis and circulatory standstill in capillaries and small vessels. Already within 5–10 min the vascular contractions were prominent and the mast cells were seen to be granulated (Fig. 4a). These reactions seemed more intense than in the deposit experiments. One could also clearly see thickening of the venular walls. The mast cells could be distinguished without intravital staining. If the toluidine blue stain was used one could see that most of them had not ruptured, but were optically more dense.

The drug was washed away with isotonic saline after 15 min. A slight improvement of the circulation occurred, followed by gradually re-established flow in some vessels. The local contractions were relaxed (Fig. 4b, c). However, the majority of the capillaries and other small vessels remained plugged and out of action for the rest of the observation time.

Long term experiments

Gross examination of the injection sites on the ears of the rabbits was made on day 8. In all sites where dequalinium had been injected there was oedema and a reddish discolouration of the skin, indicating an inflammatory reaction. There were no signs of inflammatory reactions where distilled water had been injected.

The microangiograms showed vascular proliferation with newly formed small vessels and several large tortuous venules in the dequalinium areas. Such vessels are typical of the second stage of an inflammatory reaction (Lundskog *et al.*, 1968). There was a difference in the intensity of reactions between the various concentrations of dequalinium (Fig. 5b, c), but no attempt was made to quantitate them. The solvent, distilled water, provoked only a very slight reaction (Fig. 5a).

DISCUSSION

The aim of treatment of injured tissue is to limit the extent of injury and provide optimum conditions for healing.

Irrespective of the type of injury, such as skin infection or mechanical trauma, the tissue should be treated and handled with great care after injury and during healing. Systematic studies of respiratory gas tensions in healing tissues have shown that oxygen supply is critically low in some parts of injured tissues (Hunt *et al.*, 1967). Reduction of the nutritive blood flow through a wounded area for hours by the application of an antiseptic may very well be crucial to the viability of the tissues (Brånemark *et al.*, 1967). Additional traumatization of tissues may even facilitate the development of bacterial infection if performed during the few hours after introduction of pathogenic bacteria (Miles, Miles & Burke, 1957).

Even the highest concentration of dequalinium tested, 1:1000, is below that of commercial formulations. In the present study it produced severe microvascular damage when administered to the hamster cheek pouch, without any detectable restitution within 5 h. Lower concentrations, 1:10,000 and 1:40,000, caused less severe but still substantial microcirculatory disturbances in a biphasic sequence. The immediate vascular reactions with haemolysis and reversible circulatory standstill can be attributed to the vehicle (distilled water). When circulation recovered, the signs of local vascular injury, as manifested by white emboli and white blood cells adhering to and sliding along the vascular walls, were more pronounced and lasted longer in the dequalinium experiments than after the vehicle alone. The increased vascular permeability, as demonstrated by the escape of protein-bound Evans blue into the extravascular space, indicates an injury to the endothelial blood–tissue barrier. Further evidence of dequalinium's tissue damaging effect, observed in increasing degree with rising strength of the antiseptic, are segmental vascular contractions, and detectable changes in mast cell morphology.

The microvascular effects of dequalinium are not specific for this agent or for other quaternary ammonium compounds. This laboratory has found similar tissue effects from, for example, other invert soaps such as benzalkonium chloride and cetylpyridinium chloride (Brånemark *et al.*, 1967), and following roentgen contrast media and local anaesthetics (Sörensen, 1972, Sörensen & Nilsson, 1972).

Microangiography of the rabbit ear demonstrated an increased vascularity with an abundance of newly formed vessels as compared to controls. The picture is similar to that seen in healing wounds. The findings with this experimental model confirm and extend the findings from the vital microscopy studies of dequalinium's acute tissue effects.

In the exploration of possible adverse effects from topical preparations the interest has hitherto been focussed on systemic toxicity and allergenicity at the expense of local tissue effects. The lack of generally accepted methods for assessing dermal toxicity is probably partly responsible and makes comparisons between new compounds and clinically well-tried remedies difficult. A thorough investigation of the possible tissue-irritating effect of new chemicals may reveal even weak irritants and create possibilities of taking adequate precautions before their use in humans. As tissue injury is manifested by microcirculatory disturbances, we suggest that intravital microscopic study of the cheek pouch of hamsters could be included in the screening of new compounds. The microvascular dysfunction following local application of dequalinium chloride seems to be relevant to the pathogenesis of the necrotic lesions in man. Further investigations of its influence on wound healing are in progress.

ACKNOWLEDGEMENT

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Plasma vitamin A levels in the dyskeratoses

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SUMMARY

No significant difference was found between the plasma vitamin A levels of patients with dyskeratoti skin disease and those of comparable control subjects.

A recent survey of plasma vitamin A levels (Mier & van den Hurk, 1974) showed no abnormality in the vitamin A status of adults suffering from any of the common skin disorders. Particular interest, however, has been attached to the role of vitamin A in the dyskeratoses, partly because the lesions of certain of these conditions bear some resemblance to those resulting from vitamin A deficiency (Moore, 1957a), and also because of the occasionally dramatic response of the dyskeratoses to vitamin A therapy (Cairns, 1972).

We have now measured plasma vitamin A levels in a group of such patients. Our results are presented in this communication.

MATERIALS AND METHODS

All patients with diagnoses of Darier's disease (keratosis follicularis), ichthyosis or pityriasis rubra pilaris who had attended this Department during the past 10 years were recalled by letter. The overall response was about 30%, yielding a total experimental group of thirty-five subjects. Their distribution according to diagnosis, sex and age is shown in Table 1. Specimens of blood were collected between 8 a.m. and mid-day; the plasma was separated and stored at −20°C prior to analysis. The composition of the control group and the normal range for vitamin A levels have already been reported (Mier & van den Hurk, 1974).

Vitamin A was measured fluorimetrically. Details of the technique were exactly as described previously (Mier & van den Hurk, 1974).

RESULTS

The mean plasma vitamin A level for the experimental group as a whole was 57·1 μg/100 ml, compared with a mean value of 54·1 μg/100 ml for the control group. Levels in the individual patients are illustrated in Figure 1.

Wilcoxon rank testing revealed no significant difference between any of the experimental groups and the control group.

TABLE 1. The composition of the experimental group according to age, sex and clinical status

Group	No.	Male	Female	Age (Mean and range, years)
Darier's disease	8	6	2	29·6 (23–47)
Ichthyosis (all types)	21	8	13	33·8 (16–73)
Pityriasis rubra pilaris	6	5	1	41·7 (18–62)

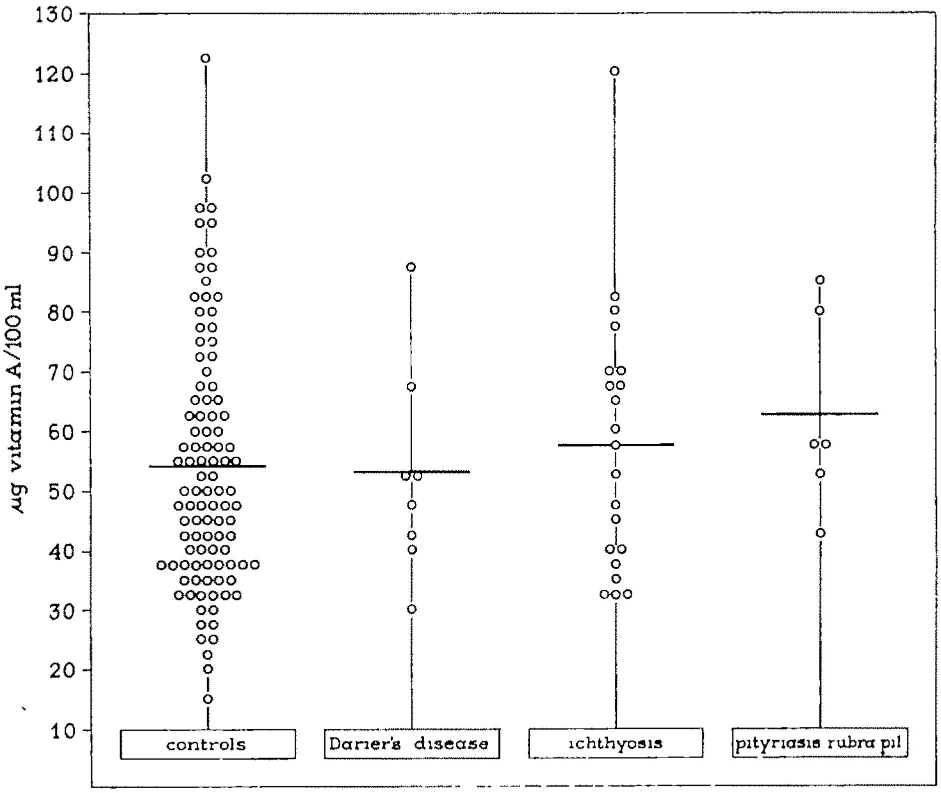

FIGURE 1. Distribution of plasma vitamin A levels according to clinical diagnosis. The values for the control group are taken from Mier & van den Hurk (1974). Means are indicated by horizontal bars.

DISCUSSION

Previous reports of vitamin A status in dyskeratotic skin disease have been adequately reviewed by Moore (1957b). In general these are singularly lacking in agreement. This difference of opinion probably stems largely from the unsatisfactory nature of the colorimetric assays which have been employed for vitamin A measurement. Additional confusion has resulted from some previous claims being based on insufficient data; in view of the wide range of values found in normal subjects, it is clear that relatively large groups are needed for any meaningful generalization.

Our present results, using a specific fluorimetric assay procedure and sufficient subjects to permit statistical evaluation, make it clear that there is no correlation between vitamin A deficiency and spontaneous dyskeratotic disease. It seems probable, therefore, that any therapeutic value of vitamin A or its derivatives results from a mechanism other than its normal utilization as a co-factor, a conclusion which is compatible with the non-physiological doses required to effect a clinical improvement in these disorders.

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The bacteriological ecosystem of the skin of children in an African tropical environment (Tanzania)

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SUMMARY

Using a standardized method for sampling, the aerobic microbial flora of the skin of ninety-five children in Dar es Salaam, Tanzania, was studied qualitatively and quantitatively. The subjects comprised three groups; group A, thirty-seven normal infants (below the age of 1 year); group B, twenty-nine infants with various bacterial skin lesions; and group C, twenty-nine nursery school children between 4·5 and 7 years.

The following observations were made:

(1) The quantitative results indicated; (a) great variation between individuals, which was more marked among the females; (b) lower bacterial counts in infants than in older children; (c) counts were not affected by the presence of infection or state of cleanliness; (d) there were some differences between females and males and between different ethnic groups.

(2) Qualitatively, the types of organisms isolated were similar to those reported elsewhere. There were differences in carrier rates of many organisms but these were most striking with *Staphylococcus aureus* and *Staph. albus*.

It is concluded that there are great differences in carrier rates between persons in a tropical African environment and those studied elsewhere. These differences may be due to environmental, geographical or ethnic factors. Such observations call for more studies in this area to establish the normal flora.

The density of the bacterial population of the skin is not known exactly for anywhere in the world (Hurst, 1965). Many workers have employed different methods to investigate the skin flora and as a result greatly varying figures have been published for similar sites of the body (Arnold, 1942; Riebel *et al.*, 1967). The colonial counts for any part of the body probably give a fairly accurate estimate of the total bacterial population. Although there may be several variables affecting the results of different investigators, figures obtained using a constant technique may be adequate for comparative studies.

This paper was presented to the Second International Conference on Basic Medical Sciences in Africa, Khartoum, 9–17 February 1974.

Work done in different parts of the world has shown qualitatively the types of microbes normally found on the human body (Williamson, 1965; Somerville, 1969). The carrier rates for many bacteria have shown great variations. The qualitative and quantitative differences might be attributed to environmental conditions, age, sex, race or other, unknown, factors.

We therefore carried out a quantitative and qualitative study in Dar es Salaam, Tanzania, in order to find out the bacteriology of the skin in a tropical African environment and to establish our own standard procedures applicable in the study of skin flora in children.

METHODS

Area and Subjects of Study

The study was conducted in the city of Dar es Salaam, which is at sea level, at the time when mean humidity and temperature were 75% and 26±8°C respectively.

Sixty-six African children attending immunization clinics in six different localities of the city and twenty-nine nursery school children were studied. The children attending immunization clinics were all infants under the age of 1 year whereas the nursery school children were aged between 4·5 and 7 years. In all groups, both sexes were equally represented. The infants were divided into two groups; group A comprised thirty-seven normal infants with no evidence of skin disease, while group B comprised twenty-nine children with various bacterial skin infections. Group C were twenty-nine nursery school children with no skin disorders. The state of cleanliness was recorded as clean, fair or dirty after inspection of the whole body and clothes.

Bacterial Studies

Samples were taken from the nose, axilla, forehead and shin of each child. Using a buffered peptone-water moistened swab, an area of 6 cm^2 on the three skin surfaces was swabbed in a circular manner, ten times in one direction and ten times in the opposite direction taking about 30 s. The nasal swabbing was twice in one circular rotation and twice in the opposite direction. The technique was standardized by one of us who did the swabbing throughout the study.

The swab was then placed in a McCartney bottle containing 5 ml of buffered peptone-water and immersed immediately in an ice-filled flask. The specimens were processed in the laboratory 2–3 h after collection. The McCartney bottle was vigorously agitated on a Fisher Mini shaker for about 30 s. A loopful of the suspension was evenly spread on 9 cm blood agar and tellurite agar plates. A standardized 10 μl loop was employed for specimens from the face and shin but because of the heaviness of growth from nose and axilla a 1 μl loop was used. The blood agar plates were incubated aerobically for 18 h before being counted then reincubated for a further 24 h for identification of the organisms. The tellurite plates were incubated for 48 h and suspected *Corynebacterium diphtheriae* colonies were identified. Identification of all organisms was based on conventional methods described by Cowan & Steel (1970). The count was expressed as: Number of colonies × 1000 × 5/6 for nose and axilla and Number of colonies × 100 × 5/6 for shin and forehead. The figures obtained represent the number of viable aerobic bacteria per square centimetre of that area of the body recovered on the cotton swab. This method yields semiquantitative results.

RESULTS

Table 1 shows the quantitative results for the three groups of children. Because of great individual variability our figures did not fit a Poisson curve and as a result we have adopted the square root transformation for all our statistical analyses. The bigger figures represent the straight means with

TABLE 1. The mean colony counts per square centimetre

Site	Normal infants		Infants with lesions		Nursery school children	
	Means	s.d.	Means	s.d.	Means	s.d.
Nose	343,556	$7·5 \times 10^{5}$	353,848	$4·5 \times 10^{5}$	550,128	$6·2 \times 10^{5}$
MSRT	5,126		5,293		7,859	
Face	4,416	$5·8 \times 10^{3}$	9,143	$1·7 \times 10^{4}$	25,673	$1·3 \times 10^{5}$
MSRT	63		130		367	
Axilla	156,469	$3·9 \times 10^{5}$	211,820	$3·5 \times 10^{5}$	9,745	$3·7 \times 10^{5}$
MSRT	2,316		3,148		3,568	
Shin	6,690	$1·5 \times 10^{4}$	7,610	$1·7 \times 10^{4}$	1,872	$3·3 \times 10^{4}$
MSRT	96		108		26	

MSRT = Means of the Square Root Transformation.

their standard deviation (s.d.) whilst the smaller figures are the means of the square root transformation.

The nose carried the most bacteria, however there was no significant difference between the counts in the nose and axilla. The two sites are not strictly comparable as the nose had a smaller area swabbed for a fewer number of times than the axilla. On the other hand both the nose and the axilla had a significantly higher count than the forehead or the skin ($P = 0·001$).

It is notable that infants with bacterial skin disorders had more bacteria on all areas investigated than did normal infants but the differences were small and not significant. The nursery children had a significantly higher count on the face than did both groups of infants; their axillae also carried more bacteria but the difference was not significant. The only area where the infants had a significantly higher count than the nursery children was the shin.

Table 2 shows the mean square root transformation figures for the faces and shins in relation to sex, race and state of cleanliness. The male infants had more organisms than the females on the face but both sexes had equal quantities on the shin. The African children had significantly higher counts on the face and shins than the Asian children. The state of cleanliness did not influence the bacterial counts on either the face or the shin.

TABLE 2. Mean square root transformation count for sex, race and cleanliness

Sex	No.	Face	Shin
Males group A	17	65·5	103·0
Females group A	20	34·6	110·0
Males group C	15	35·0	21·4
Females group C	14	10·5	19·6
Race			
Africans	16	32·8	28·9
Asians	9	12·5	9·1
Cleanliness			
Clean	4	76·5	170·0
Fair	22	70·6	48·9
Dirty	12	76·0	167·0

Qualitatively, the types of organisms isolated are shown in Table 3. Six genera of gram positive organisms and four genera of gram negative organisms were isolated. The most frequent organisms were *Staph. aureus*, diphtheroids, Sarcina and *Staph. albus*. The four groups of organisms occurred with varying frequency on all four parts of the body investigated. There was a significantly higher carrier rate of most organisms in the infant groups than the nursery school children (Table 4). There was no significant difference between the carrier rates of normal infants and infants with bacterial infections.

TABLE 3. Carrier rates of different organisms in percentages

	Nose			Axilla			Forehead			Shin		
Organisms	A	B	C	A	B	C	A	B	C	A	B	C
Staph. aureus	65	58	44	92	88	75	84	10	27	65	75	14
Diphtheroids	68	51	65	30	24	10	19	7	14	16	14	7
Sarcina	16	7	3	54	41	14	76	14	44	78	54	61
Staph. albus	22	17	10	30	54	31	32	7	7	30	31	10
Neisseria	41	61	37	0	0	0	0	3	0	0	0	0
Pneumococci	30	27	14	0	0	0	5	7	0	0	0	0
Bacillus sp.	12	3	10	3	10	17	5	0	34	5	10	61
Gm. neg. rods	3	7	0	10	14	0	3	3	0	11	0	0
Micrococci	0	7	0	8	7	3	16	7	0	0	7	0

A, Normal Infants—37; B, Infants with skin lesions—29; C, Nursery School Children—29.

TABLE 4. The levels of significant carrier rates infants v nursery school children

Organisms	Site	$P<0.001$	$P<0.01$	$P<0.05$
Staph. aureus	Nose	—	—	Infants > Children
	Axilla	—	Infants > Children	—
	Face	Infants > Children	—	—
	Shin	Infants > Children	—	—
Sarcina	Nose	—	—	Infants > Children
	Axilla	—	Infants > Children	—
	Face	—	—	Infants > Children
Staph. albus	Face	—	Infants > Children	—
	Shin	—	—	Infants > Children
Bacillus sp.	Face	—	—	Infants < Children
	Shin	Infants < Children	—	—
Diphtheroids	Axilla	—	—	Infants > Children

In terms of individual organisms the pattern of carrier rates was as follows:

Staph. aureus. The carrier rate for this organism ranged from fourteen to ninety-two. The axilla had the highest rate followed closely by the face; the shin had the lowest carrier rate. There was a significant difference between the infants and the older children and this was more marked on the uncovered parts of the body.

Diphtheroids. The carrier rate was highest in the nose of infants (68%) and lowest on the shin of the older children (7%). Except for the nose, the infants' carrier rate was significantly higher than that of the nursery school children. The organism occurred more frequently in the moist areas than on exposed dry parts.

Sarcina. The carrier rate was highest on the shin of infants (78%) and lowest in the nose of nursery school children (3%). This is the reverse of the results for *Staph. aureus* and diphtheroids in that Sarcina occurred more frequently on the dry exposed parts of the body.

Staph. albus occurred most frequently in axillae of infants (54%) and least on the face of nursery school children (7%). Although *Staph. albus* species had a lower carrier rate than *Staph. aureus*, they had a similar pattern of distribution.

Neisseria species and Pneumococci. Both groups of organisms were found mostly in the nose (37–61% and 14–30% respectively) but a few infants had these organisms on the face. The carrier rate for both organisms was higher in the younger children than the older.

Bacillus species. The carrier rate varied from nil on the face of infants to 61% on the shins of nursery children. This is the only organism whose carrier rate was significantly higher in the older children than in infants. It was more common on the exposed parts particularly the lower parts of the body.

Gram negative bacilli were found on all parts of the infants with a carrier rate ranging from 3–14% but none on the bodies of older children.

Micrococci were found mostly in infants and on all parts. Only one nursery school child had the organism in the axilla.

Group A beta haemolytic streptococci were isolated from two infants; once from the shin of a normal infant and the other from the face of an infant with a streptococcal scalp impetigo.

Corynebacterium diphtheriae was isolated only once, from a normal infant's shin. This particular infant had high counts of all organisms all over the body.

DISCUSSION

Although it has not been possible to summarize all the information obtained from this study, a number of interesting observations regarding the aerobic bacterial flora of the skin of African children in a tropical environment have been made. Quantitatively it is clear that, as in many other studies (Arnold, 1942), our study is subject to deficiencies as there is no perfect technique for assessing accurately the total population on the skin. Williamson's method (1965) is the best but this is only applicable to willing and cooperative subjects and could therefore not be used in this study of infants and children.

The total counts on individuals had a wide scatter and therefore big standard deviations; Rebel and others (Rosebury, 1961) have made similar observations. Children's counts vary considerably more than do those of adults. For this reason it was not possible to use the original figures for statistical purposes but when we employed the square root transformation of the mean some statistically useful information was obtained.

The nasal antrum had the highest counts for all age groups. This may be attributed to the moisture and temperature within the nose which promote the survival and multiplication of micro-organisms. The second highest counts were obtained from the axilla. This is also humid and has a high pH which promotes growth of a big number of mixed flora (Kligman, 1963). The counts for the forehead and shin were much lower than for the axilla and nose, because the former are uncovered and relatively drier and presumably unfavourable for the multiplication of bacteria.

The foreheads of the nursery school children had significantly higher counts than those of the infants. The cause for this is difficult to determine. However, it is likely that the faces of nursery school children are more moist than infants' faces because the sweat glands of the older children are more developed and they sweat more as a result of their physical activities. Although the pH was not determined, it is not likely to have had any influence since the pH of the face is fairly constant after the first week of life (Noble, 1968). The absence of any difference between the faces and shins of infants suggests that they are equally moist and maintain similar temperatures as well. However, there was a significant difference between the faces and shins of the older children suggesting that their shins are less moist and are cooler than their faces and so do not support survival and/or multiplication of many micro-organisms.

The counts on infants with bacterial skin infections were always higher than the counts for the normal infants but the difference was not significant. This is an unexpected finding since bacterial lesions contain large numbers of organisms which, from time to time, might be shed on to the rest of body surface. It has also been demonstrated that any skin disorder is likely to result in higher bacterial counts at sites other than the lesion (Noble, 1966). It could also be reasoned that the carriage of large numbers of pathogenic or potentially pathogenic organisms could predispose to bacterial infections. This study has shown that neither of the two expected findings was associated with bacterial infections of the skin. The majority of the lesions however had a hard crust which could have prevented the organisms from being spread on the body surface.

Price (1938) has shown that the amount of surface flora is influenced by sex on some parts of the body. On the sites we investigated the only sex difference observed was that male infants had higher counts on the face than female infants. Explanations for the differences at this age have not been elucidated. The absence of any significant difference for different states of cleanliness is not surprising since cleanliness affects only the transient flora and has a negligible effect on the resident flora. It has been demonstrated by Evans *et al.* (1950) that thorough washing or bathing has no permanent effect on the resident flora and that going without a bath for 6 days does not increase the total counts.

Finally, one other aspect considered was whether there is any racial differences in total counts. The only difference detected was on the forehead where African children had a higher count than children of Asian origin. The numbers of Asians was however small and further studies to verify this are contemplated.

Qualitatively, the isolates were the well known genera of bacterial flora on the human body (Williamson, 1965). A few common organisms were however not isolated in this study. This could be either a result of the method used, since it could not detect less than seventy viable organisms/cm^2, or that these organisms did not occur in these particular groups studied. Table 5 shows our isolates from the forehead for normal infants and children and compares them with a similar study of Somerville (1969) to show the diversity in types of organisms and carrier rates that could exist from one place to another.

There is a big difference in the two studies in the carrier rates of *Staph. aureus* and *Staph. albus* on the forehead. Contrary to the usual findings that the carrier rate for *Staph. albus* is higher than for *Staph. aureus*, our findings were completely the reverse. Secondly, our infants carried a greater variety of organisms than older children whereas Somerville's results are the opposite. Some other important differences observed between these two studies were of carrier rates in relation to age, sex, and parts of the body. There was a similar trend in carrier rates of gram positive organisms and an opposite trend of gram negative organisms as shown in Table 6. Gram negative rods being more common among infants than older children in this study could be explained on the fact that the infants are rarely covered with nappies and as a result they tend to soil themselves with coliforms. Again, we found that gram negative rods occurred more frequently in association with diphtheroids. This is of marked contrast to the belief that the gram negative rods are inhibited by presence of diphtheroids (Marples, 1969).

TABLE 5. Percentage carrier rates of organisms isolated from forehead

	Infants		Children	
Organisms	Somerville (1969)	Nsanzumuhire *et al.* (1974)	Somerville (1969)	Nsanzumuhire *et al.* (1974)
Staph. aureus	26	83	39	27
Staph. albus	96	32	98	7
Streptococci	66	7	51	0
Diphtheroids	19	19	50	14
Enterics	6	0	0	0
Gm. neg. rods	0	3	50	0
Sarcina	0	76	66	44
Bacillus sp.	0	5	57	34
Neisseria	4	3	25	0
Pneumococci	0	5	0	0
Yeasts	12	0	8	0

TABLE 6. A comparison of Somerville 1967 and Nsanzumuhire *et al.*, 1974

Organisms	Site	Somerville, $P = 0{\cdot}001$	Nsanzumuhire
Sarcina	Forehead	Females > Males	Females > Males ($P = 0{\cdot}5$)
Diphtheroids	Forehead	Children > Infants	Children > Infants ($P = 0{\cdot}3$)
Gram. neg. rods	Whole body	Children > Infants	Infants > Children ($P = 0{\cdot}001$)
Neisseria	Whole body	Children > Infants	Infants > Children ($P = 0{\cdot}1$)

Streptococcus pyogenes group A was common neither on the skin of normal children nor on that of children with bacterial infections. Although Allen, Taplin & Twigg (1971) have shown that 58% of men with severe streptococcal pyoderma carried the organism in at least one other normal area, we found only one out of ten children with streptococcal pyoderma had the organism on normal skin. This may be due to the difference in types of lesions. Our pyodermas were mostly of a mild impetiginous type with a hard crust on the surface so that the organisms were hidden from the skin surface.

Corynebacterium diphtheriae was also rare on the normal skin. The organism was found only once on the skin of a normal infant and not from any other group, although it occurs in about 35–50% of all pyoderma cases in this area (Mhalu, 1973; Mhalu & Lindqvist, 1974). The reason for its rarity is similar to that for *Strept. pyogenes*, that the hard crust of the lesion hides it from surface. It is concluded that this organism is either scanty on the skin or that it is not part of the normal resident skin flora and is a secondary invader of lesions (Mhalu & Lindqvist, 1974).

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Pharmacology and Treatment

Treatment of solar keratoses with a 5-fluorouracil and salicylic acid varnish

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SUMMARY

In an attempt to avoid the side-effects of treating solar keratoses with 5% 5-fluorouracil (5-FU) ointment, a new pharmacological varnish containing 5% 5-FU and 5–10% salicylic acid in collodium was tried. Twenty patients with such lesions were treated. A drop of the varnish was applied on each lesion every 3 weeks. Only one to five applications on facial lesions were necessary to obtain apparent cure in all patients. Lesions reappeared in four patients, but were cured after a second and similar treatment. The keratoses of the hands were more resistant and needed seven applications in one patient and nine in the other.

The topical application of 5-fluorouracil (5-FU) ointment to multiple solar keratoses has proved to be a very effective treatment. In an attempt to control and minimize the sometimes considerable discomfort caused by this treatment, the Scientific Department of Hoffman-La Roche Laboratories was asked to prepare varnishes containing 5% 5-FU, and a variable concentration of salicylic acid (SA).

MATERIALS AND METHODS

The excipient of the varnish is composed of equal parts of Collodium and Collodium elasticum, as indicated in the *Pharmacopée Helvétique* VI. The composition of the Collodium is; pyroxilinum, 4.0 g; ethanol, 30.0 g; ether, 66.0 g. The composition of the Collodium elasticum is; Collodium, 97.0 g; castor oil, 3.0 g;

The pyroxilinum or colloxylinum is a mixture of dinitrocellulose with tiny quantities of trinitrocellulose wetted with 94% ethanol.

Twenty patients with solar keratoses were treated, eight men and twelve women, whose ages varied from 42 to 79 years. One female patient had lesions on the hands and a male patient had multiple lesions on the hands (Fig. 1) and face. The keratoses on the hands of both patients were very thick, there being about ten on each hand. Seven patients had only one keratosis on the face, one had two, one had three, one had five and nine had between ten and twenty. The lesions had been present for periods from 1 month to 20 years.

Six patients had been treated with 5% 5-FU ointment 1–14 months previously. Some of the lesions

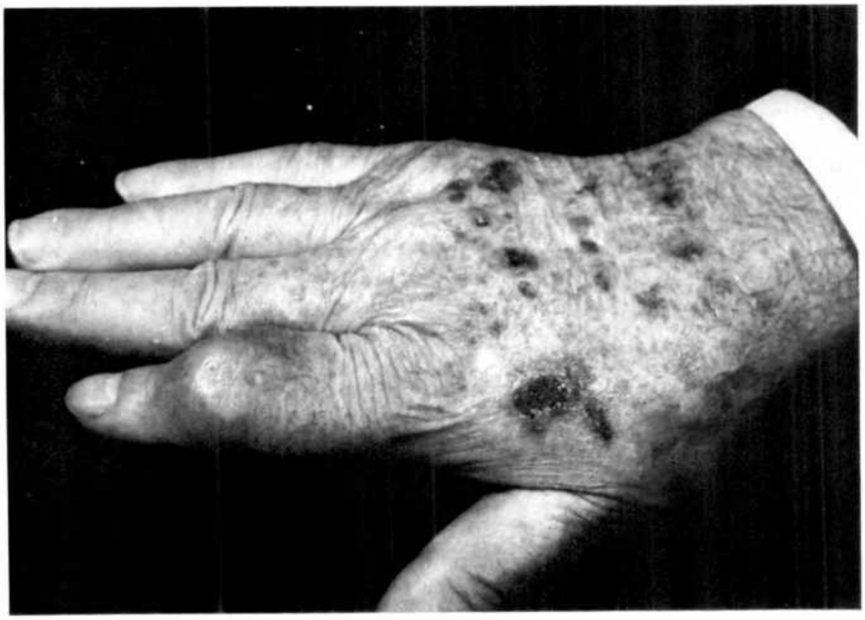

FIGURE 1. Thick senile keratoses on the dorsum of the hand of a man.

were new, others were probably recurrences. One patient had been treated with dry-ice. The remainder had not received any previous treatment.

One drop of the varnish was applied to each lesion by the author. As the varnish adheres so firmly to the skin that it is shed 2–3 weeks after application, only one treatment was carried out every 3 weeks. The criterion used to determine apparent cure was the disappearance of the lesions as observed by sight and touch.

Varnishes containing 5% 5-FU combined with 5%, 8% or 10% SA were applied on different patients. A comparison with a SA varnish without 5-FU was not considered necessary because it is well known that SA preparations do not cure such lesions. A further trial with a 5% 5-FU varnish without SA will be carried out.

RESULTS

The varnish dries in a few minutes and forms a cosmetically acceptable white film. No dressing is necessary. The varnish adheres so well to the skin that it is not shed with bathing or swimming, but excessive sweating must be avoided.

All the lesions were cured with minimal side-effects, after which the skin remained smooth and looked normal. The various varnishes containing different percentages of SA all gave similar results, and the 5% 5-FU and 5% SA varnish is the one now used.

For the lesions on the face, the number of applications varied between one and five; five patients received one single application, five patients two applications, two patients three applications, five patients four applications and two patients five applications. Some patients appeared irregularly for treatment, coming every 2–4 weeks. They were cured as well.

No patient suffered from traumatic dermatitis or photosensitization. Immediately after the application some patients reported a slight burning sensation that lasted a few minutes. This was the only side-effect observed.

Five patients did not come for follow-up, ten remained well after 2–10 months. One of the multiple keratoses of one male patient recurred. Three other patients developed new lesions, but it was not possible to determine with certainty if they were true recurrences or independent new lesions. All these new lesions were again treated with the same varnish and all were cured, with one, three or five applications. This time, one more drop was applied after the apparent cure.

The thick keratoses on the hands of the two patients were cured after seven and nine applications respectively (Figs. 1 and 2).

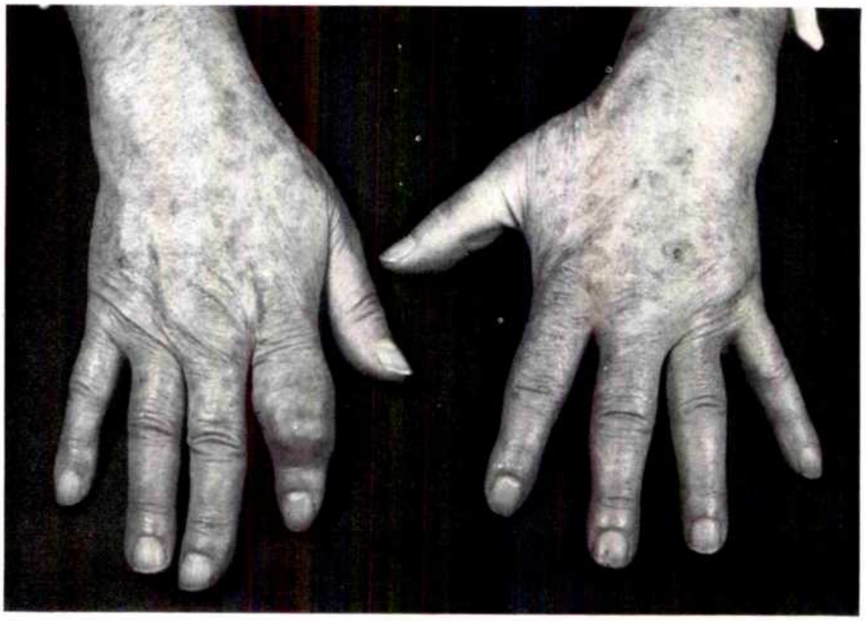

FIGURE 2. The lesions cured after seven applications of the varnish.

DISCUSSION

The action of the 5-FU and SA varnish on the solar keratoses greatly surpassed the author's expectations. The side-effects that typically occur with the ointment were expected to be reduced with the varnish but not to such a degree. In fact, they were practically non-existent.

The varnish was found to be superior to the ointment in many aspects. It cured the keratoses with a small number of applications, generally one to four, exceptionally five. This difference is probably due to the intimate and prolonged contact between the compound and the lesion. The main advantage is the simplicity and comfort of application and the absence of side-effects.

Four patients had new lesions after apparent healing. The new (or recurrent) lesions were cured as well with the same method of treatment.

Solar keratoses of the hands show much more resistance to the 5-FU ointment. The advantage of the varnish was demonstrated by the willingness with which the two such patients responded to the treatment, for even though it involved many applications (seven in one case, nine in the other), they did not have to endure the slightest discomfort or side-effects.

To avoid recurrence, one more application after apparent cure is advised.

The action of the varnish on solar keratoses is superior to that of the ointment, except when a rapid cure is desired or when there are dozens or even hundreds of keratoses, some of them invisible. In these cases it is not practical to treat the whole number with individual drops of varnish. Under such conditions, there should be an initial treatment with 5-FU ointment to cure all or most of the lesions, and if some remain, as they generally do, the varnish could be used subsequently.

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5-Fluorouracil in the treatment of common warts of the hands

A DOUBLE-BLIND STUDY

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SUMMARY

A double-blind study was carried out to compare the action of a 5-fluorouracil and 10% salicylic acid varnish with that of a 10% salicylic acid varnish on common warts. Fifty per cent of the lesions disappeared with the combined preparation and only 4% with the varnish containing salicylic acid alone.

The presence of warts on the hands of 45.8% of the female workers of a fowl slaughterhouse is described.

There are many treatments for common warts. Most are ineffective, many are painful and some are dangerous. The therapeutic activity of topical 5-fluorouracil (5-FU) on benign and malignant tumours of the skin suggested this study of its effect on warts.

Before the start of the present study a few cases of warts of the hands were treated with a 5% 5-FU ointment without occlusion. No patient showed improvement. Then six patients with multiple warts of the hands were treated with the same ointment, but with occlusive dressings in addition. Only one patient was improved after many weeks of treatment.

The Scientific Department of Hoffman-La Roche Laboratories was then asked to provide a varnish with 5% 5-FU associated with 10% salicylic acid (SA) and a similar varnish with only 10% SA for comparison.

MATERIAL AND METHOD

The excipient of the varnish is composed of equal parts of Collodium and Collodium elasticum (Pharmacopée Helvétique VI(12)). The varnishes stick so firmly to the skin that they cannot be removed by washing of the hands or by domestic work.

In a fowl slaughterhouse in Lisbon the percentage of female workers suffering from warts on the hands was high. Twenty-seven women out of fifty-nine had such lesions (45·8%). The patients were observed and the number, localization and dimensions of the individual lesions were recorded.

The twenty-seven patients had 122 warts, sixty-five on the right hands and fifty-seven on the left hands.

The head of the slaughterhouse, a veterinary surgeon, was asked to apply daily, at the end of the working day, one of the varnishes to the lesions on the right hands and the other to the lesions on the left hands of all patients. The varnishes were in flasks labelled A and B. The author did not know which varnish was being applied to each side.

Every 2 weeks the differences in the size and number of the warts were recorded. The trial continued for 9 weeks.

RESULTS

There were sixty-five warts on the right hands to which the SA varnish was applied and fifty-seven lesions on the left hands to which the 5-FU and SA varnish was applied.

On the right side 4% of the lesions disappeared, while on the left 50% of the lesions disappeared. The warts that were not cured decreased in size on both sides, but more so on the left side than on the right.

DISCUSSION

The presence of numerous warts on the hands of people who deal with dead animals has rarely been referred to. Marshall (1964) noted it in fishermen and fish-handlers and Bosse & Christophers (1964) in veterinary surgeons and butchers. No references could be found to warts occurring in those handling fowls, alive or dead.

References in the literature to the treatment of warts with topical cytostatics are scanty. Duthie & McCallum (1951) treated plantar warts, first with occlusion under Elastoplast for 4–6 weeks and cured 60% of the warts. The cases that resisted this treatment were subsequently treated with 25% podophyllin in liquid paraffin. Using both methods they cured thirty-seven of forty cases. They stressed that 'Podophyllin can produce a severe primary chemical dermatitis and it is also a powerful sensitizer'. For this reason they avoided its use on the hands.

Marker & Reymann (1967) treated plantar warts first with a strong keratolytic under occlusion. The patients were then divided into five groups and treated with the following five topical cytostatics: (1) a gel containing 10% of a 'semisynthetic' podophyllin derivative, podophyllotoxin-beta-D-benzylidene-glucoside (SPG, Sandoz); (2) 10% 5-FU in vaseline; (3) an ointment with equal volumes of demecolcine (Colcemid, Ciba), N-deacetyl thiocolchicine (Thiocolciran, Roussel) and Methotrexate; (4) vaseline with 20% p-(methyl-hydrazinomethyl)-N-isopropyl-benzamide-hydrochloride (Natulan, Hoffman-La Roche) and (5) vaseline containing 0·02% vinblastine sulphate (Velbe, Eli Lilly). An average cure rate of 51·5% was obtained. There was no statistically significant difference between the outcome of the treatment with the five cytostatics used or between them and occlusion with the strong keratolytic.

McAusland (1935) and Batemann (1942) cured 50% of warts using only occlusion without any drug.

Harmann *et al.* (1972) stated that 'podophyllin is usually ineffective in warts of other types but good results have been obtained in some plantar warts after preliminary removal of callus'.

Brandão (1965) treated twenty-one patients with warts, thirteen of them on the hands, with 0·7% cyclophosphamide ointment (Endoxan, Asta-Werke) under occlusion. He cured sixteen patients. The duration of the treatment was between 2 and 7 weeks.

Peryassú *et al.* (1969) treated six cases of warts with 5% 5-FU ointment under occlusion. After 10–33 days the lesions were compact and were removed with a curette. They all healed. Midana & Leigheb (1970) treated ten cases with 5% 5-FU ointment under occlusion. Only two patients were cured. Thivolet *et al.* (1969) treated four warts on the hands and one plantar wart with the same ointment. No patients were cured.

The author's experience with 5-FU ointment agrees with that of Midana and Thivolet. The use of occlusion and curette by Peryassú *et al.* does not permit comparison.

It may be doubted whether the results achieved under occlusion have anything to do with the topical cytostatics used. Marker & Reymann (1967) believed that the actual cause of cure was the occlusion. It has been shown that physical occlusion reduces the normal as well as the psoriatic epidermal mitosis rate (Fisher & Maibach, 1972).

In this double-blind study the action of a strong keratolytic (10% SA) was compared with the action of this same substance in association with 5% 5-FU, both in the same excipient, and without the use of an occlusive dressing.

The difference obtained in cure rate is remarkable. The 50% cure rate obtained equals that found using methods in which occlusion was used. The 4% cure on the right side probably corresponds to the spontaneous disappearance rate in people who work with wet hands all day.

In this study the cures cannot be attributed to any physical effect. The varnish occludes only partially and the films over the lesions on both sides were indistinguishable and remained in place for the same length of time.

This trial shows that the 5-FU varnish is apparently more active than the cytostatics previously used but it must be stressed that to cure 50% of lesions is not satisfactory therapeutically. A better cure rate may be dependent on better penetration.

In an effort to improve the penetration of the drug, a pilot trial using a 5% 5-FU, 10% SA and 3% trypsin varnish was subsequently carried out. The results were no better.

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A controlled trial on the use of topical 5-fluorouracil on viral warts

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SUMMARY

A double blind controlled trial was performed on the use of topical 5% 5-fluorouracil ointment as a treatment for viral warts. The results showed the 5-fluorouracil to be better than the placebo.

MATERIALS AND METHODS

General Practitioners in the district were asked to cooperate in the trial by referring patients to an investigative clinic set up at Nelson Hospital for this purpose. Patients of any age or sex were accepted provided they had bilateral warts.

Sixty-six patients were treated, but one failed to return for follow up and one record card was wrongly completed so that sixty-four patients were assessed in the trial. There were forty children aged 5–14 years (nineteen female, twenty-one male) and twenty-four adults (sixteen female, eight male).

Matching 10 g tubes of active and dummy cream were prepared by Roche Products Ltd, and labelled by them, pairs being numbered consecutively. One tube of each pair contained 5% 5-fluorouracil (5-FU) whilst the other contained base only. One tube in each trial packet was labelled 'L' for use exclusively on lesions on the left side of the body, whilst the other was labelled 'R' for use on lesions on the right only.

Whether the active agent should be applied to the left or right side was determined in random fashion according to the tables of Fisher and Yates. A sealed code was provided. The packaged pairs of tubes were chosen from their case in random fashion by a nurse at the clinic.

Patients were seen after 2 weeks of treatment and progress recorded, and then again after another 2 weeks when final clinical assessment was made. In some cases, where warts were obviously responding but not completely cleared, therapy was continued after completion of the official trial period, but this time was not used in assessing the results of the trial.

At the initial consultation, patients were given a package containing the 2 tubes of cream as described, and were carefully instructed to use them as labelled, for lesions on the left or right. Each day a small amount of cream was placed on each wart and covered with waterproof adhesive plaster, which was left for 24 h. The area was then cleaned, dried, and the cream reapplied with more plaster covering. Patients were warned not to let it get under finger nails.

Random samples of cream were returned to the manufacturer for assay after completion of the trial and were found still to contain 5–5.49% of active 5-FU.

Completed record cards were finally sent to Roche Products Ltd, Research Department for the results to be tabulated and analysed statistically.

RESULTS

Of the sixty-four cases, active cream was used on the right side in thirty-one cases and on the left in thirty-three.

TABLE 1. Overall results

	Cleared	Improved	No change
5-FU	35	10	25
Placebo	14	11	45

Six patients on 5-FU and six on placebo had different results on two treated sites, which explains why there are seventy results for each, rather than sixty-four.

Excluding the six patients with multiple sites and different results, and only looking at clearance or no change, the result is as follows:

TABLE 2

	Cleared	No change
5-FU	29	22
Placebo	8	43

Excluding three patients where it was noted that treatment was applied in an unsatisfactory manner and who were all failures, the following is the final result:

TABLE 3

	Cleared	No change
5-FU	29	19
Placebo	8	40

This is highly statistically significant in favour of 5-FU (χ^2 equals 19·39; $P < 0.001$).

If we look at individual patients, 5-FU was better than the placebo in thirty-six and equal in sixteen. The placebo was better in twelve patients.

Detailed analysis of the results according to site, sex and age was made and showed that there were significant cure rates in all groups using 5-FU, that it was more effective in children than adults and more effective on fingers and hands than on other sites.

The major side effect was onycholysis, this having occurred in eleven patients with lesions of the finger tip or paraungual lesions. The only other side effect seen was due to plaster sensitivity or irritation.

DISCUSSION

Viral warts present one of the perpetually recurring problems in dermatological practice. Treatments are legion and have included curettage, cautery, diathermy, caustics, acids, cryotherapy, superficial X-ray therapy, ultrasonic therapy, hypnosis, podophyllin, keratolytics, and simple occlusion.

All of these methods have been shown to give a significant cure rate, but they all also have a disappointing and apparently inexplicable recurrence or failure rate, while the problem of dealing with multiple warts in the young can prove to be a formidable therapeutic challenge.

5-FU is a fluorinated pyrimidine which apparently interferes with the synthesis of DNA and of RNA to a lesser degree. Since viral warts should require a generous supply of DNA and RNA for both the virus particles as well as for the proliferating epidermal cells, it was felt that 5-FU might be a suitable agent to combat them. However it is possible that clearance of warts by some methods of treatment is due to enhancement of immune responses. Chemical treatments, in particular, may facilitate viral absorption which may then cause viral destruction by immunological mechanisms.

This method of treatment of warts with topical 5-FU was reported by Goldman, Blaney & Cohen (1962); Hursthouse (1970) reported a 67% clearance rate for viral warts in a small uncontrolled series using this method. Bunny (1973) published results of a trial of topical 5-FU for mosaic plantar warts using differing vehicles for the active agent and obtained cure rates from 46·5% to 53%.

Jaross (1970) advocated the use of topical and intralesional 5-FU in the treatment of warts especially where tissue destroying methods had been ineffective or produced adverse results. He claimed a 75% cure rate, but did not quote any trial.

The natural history and results of various methods of treatment of warts of the hands in 2006 patients, over a period of 10 years, have been reviewed by Barr & Coles (1969) who found that overall cure rates from various methods of treatment varied from 16·6 to 80·4%. Fifty-three patients of this large series received no treatment and had a spontaneous cure rate of 86·6% in the period of observation.

The results of this present trial show that topical 5-FU is significantly better than a placebo in the treatment of warts, but the overall cure rate was only about 60%. It is also a very slow method. Nevertheless, it could have a place in treating patients who have great fear of physical methods, where there is a known tendency to cheloid or pigmented scarring, in cases of repeated recurrence following other methods and in those occupationally exposed to serious secondary infection following physical removal, e.g. Veterinary Surgeons.

The author uses this method in selected cases for the above reasons, especially in cases of multiple plantar warts and for isolated lesions on fingers where plaster occlusion is simple. In fact the method is frequently specifically asked for by referring doctors who have had it used on previous patients.

ACKNOWLEDGMENTS

I wish to thank my colleagues for sending cases for investigation, the Medical Superintendent, Nelson Hospital for permission to run an investigative clinic, Roche Products Ltd for printing record cards and providing coded creams, and Dr Ian Lennox Smith for analysis of the results with comments thereon.

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Anti-viral treatment of warts

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SUMMARY

Several anti-DNA agents have been tested for their value in the treatment of warts in humans. A pilot study was carried out on patients with warts resistant to other forms of treatment. The agents used were hydroxyurea, idoxuridine and photodynamic inactivation. Idoxuridine 20% in a cream base appears to offer some possibilities as a treatment when other measures have failed.

There is good evidence that warts in humans are caused by a member of the papova group of viruses. Accidental and experimental transmission of the virus with subsequent development of warts has been recorded by many authors and their results are well reviewed by Rowson & Mahy (1967). The wart virus is a DNA-containing virus and at present there are a number of anti-DNA agents available with virucidal activity and relatively low toxicity. The purpose of this paper is to report a pilot study using some of these agents in patients with recalcitrant warts.

PATIENTS

Twenty-four patients were included, seven males and fifteen females. Their mean age was 35 years, range 15–57 years. In sixteen patients the warts were confined to the feet, eight of these having mosaic warts; in five patients the warts affected only the hands and in three patients the hands and feet were both involved. The number of warts ranged from one to twenty per patient. Duration of the infection varied from 1 year to 21 years with a mean of 4·6 years. All patients had received at least two other forms of treatment before admission to the trial.

MATERIALS AND METHODS

Three agents were used (1) Hydroxyurea 25% in dimethyl sulphoxide (DMSO) applied daily. (2) Idoxuridine (IDU) in the following forms: (a) 40% in DMSO applied daily; (b) 40% in a cream base applied daily; (c) 40% in a cream base applied daily under occlusion; (d) 20% in a cream base applied daily under occlusion; (e) 10% in a cream base applied daily under occlusion. (3) Photodynamic inactivation using proflavine 1% aqueous solution painted on the wart daily, followed by exposure to a daylight-type (Osram) fluorescent tube at a distance of 6 in for 20 min.

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With all treatment regimens the results were reviewed after 4 weeks and if there were no signs of improvement the treatment was stopped. If there were signs of regression the treatment was continued up to a maximum of 3 months. Any one patient, during the course of the trial, may have received two or even three different approaches to treatment. All warts in any given patient were treated in the same way.

RESULTS

These are shown in Table 1. Duration of treatment in the successful cases ranged from 3 weeks to 3 months, IDU being quicker in most cases with a mean regression time of 6 weeks. All the warts in any particular patient showed the same response to treatment; if regression occurred, it did so in all warts.

TABLE 1. Results of the treatment regimens showing number of patients whose warts did and did not resolve while undergoing a particular treatment

Treatment	Patients resolving with treatment	Patients not resolving with treatment	Patients treated
1. Hydroxyurea 25% DMSO	0	5	5
2. IDU 40% DMSO	3	6	9
3. IDU 40% cream without occlusion	0	2	2
4. IDU 40% cream with occlusion	2	0	2
5. IDU 20% cream with occlusion	10	3	13
6. IDU 10% cream with occlusion	0	3	3
7. Photodynamic inactivation	3	2	5
Total	18	21	39

DISCUSSION

Hydroxyurea 25% in DMSO and IDU 40% in DMSO appear to be of little value in the treatment of warts. This is perhaps the fault of the base rather than the active constituents. DMSO probably carries the agent through the keratin so rapidly that there is insufficient time in contact with the slowly replicating wart virus. For this reason a cream base was next used and later plastic occlusion with the aim of keeping a reservoir of IDU in constant contact with the wart. This idea appeared to be successful using 40% IDU and due to the high cost of this approach, lower strengths were tried. 20% IDU cream gives encouraging results but the 10% strength appears not to be effective.

Photodynamic inactivation using photo-active heterotricyclic dyes has been used successfully in herpes simplex infections (Feber *et al.*, 1971) and against a papova virus, SV40 (Wallis & Melnick, 1964). Although in the present study the results of this treatment are not entirely encouraging, it should be pointed out that three of the patients in whom it was tried had already failed to respond to IDU 20% cream; one of these responded to inactivation treatment.

In warts antiviral agents have received little attention and yet possibly they offer the best hope for a safe effective cure. That a virucidal approach did not give a 100% cure rate in the present study may either indicate that not all warts need live virus to sustain them or the agents available are not ideally suited for the purpose.

ACKNOWLEDGMENTS

I wish to thank Cambrian Chemicals who kindly supplied the IDU for this trial, E.R.Squibb who supplied the hydroxyurea and Dr A.Beale of Burroughs Wellcome who suggested the idea of photodynamic inactivation. Mr John Grayson and the staff of St Helier Hospital pharmacy were extremely helpful in preparing and distributing the preparations. Dr E.L.Rhodes kindly permitted study of her patients.

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Case Reports

Solar urticaria

A CASE WITH POSSIBLE INCREASE OF SKIN MAST CELLS

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SUMMARY

A case of solar urticaria is described showing: (1) Action spectra for late erythema (MED), late swelling and wealing with one peak of sensitivity for erythema and wealing at 405 nm. (2) No signs of porphyria. (3) Possibly increased skin mast cells. (4) Short-lived post-irradiation fibrin deposition. (5) Haemolysis. (6) Apparent suppression of urticaria with the antihistamine Incidal.

The term solar urticaria covers a poorly understood group of dermatoses, which share one feature, i.e. wealing from ultraviolet radiation (UVR) and/or visible light.

The extreme variety of action spectra (Ive, Lloyd & Magnus, 1965) led Harber & Baer (1969) to classify solar urticaria into not less than eight types.

Harber and Baer's type 6 is the one best understood i.e. erythropoietic protoporphyria with solar urticaria, whilst in other cases transfer experiments have suggested an auto-allergic pathogenesis (Illig, 1963; Harber & Baer, 1969; Pathak & Epstein, 1971).

In some cases the wealing seemed to be histamine-mediated (Ive *et al.*, 1965; Grice, Ryan & Magnus, 1970), but Sams, Epstein & Winkelmann (1969) and Ramsay, Scrimenti & Cripps (1970) could not demonstrate histamine or kinins in dermal perfusates.

The present communication deals with additional investigation on a case of solar urticaria.

CASE-REPORT

For 3 years a 48-year-old male complained of painless itching erythema of the skin immediately after a short exposure to bright diffuse daylight or direct sunshine, even through window glass. The eruption persisted for several hours. Sunbathing provoked nausea and faintness.

Exposed to more light than normal (e.g. on holiday) he noted that after several days his symptoms diminished.

His personal and family history and physical examination were negative except for jaundice 25 years before.

Phototests

Action spectra were determined repeatedly at 257, 297, 366, 405, 436 and 546 nm (Rottier, 1969).

Minimum doses were determined for late erythema, wealing (immediate swelling) and late swelling by observing the skin repeatedly after irradiation.

The abnormal reactivity of this patient's skin became apparent by wealing at 366 and 405 nm and by late erythema at 405 nm and 436 nm radiation. Up to 366 nm, the late erythema sensitivity was normal; at 546 nm, with the very high dose used, this reaction could not be provoked. The action spectra are plotted in Fig. 1.

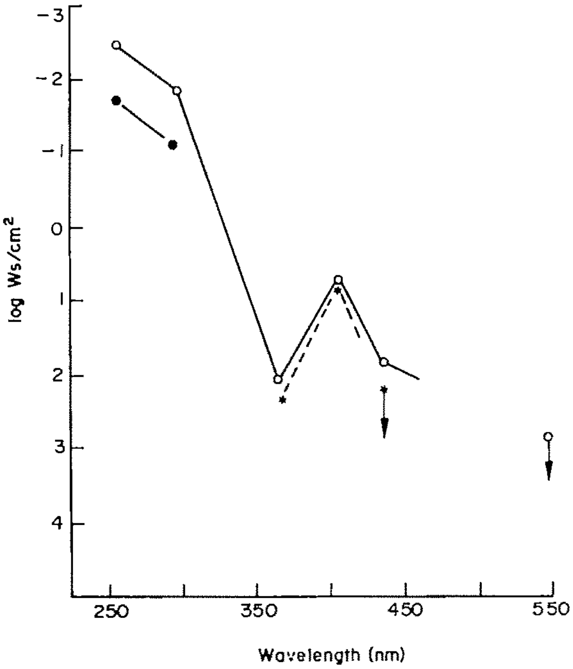

FIGURE 1. Action spectra for minimum irradiation doses (ordinate) plotted against wavelength (abscissa). Data from the skin of the back in a case of solar urticaria. ○—○, MEDs; ●—●, Late swelling at 257 and 297 nm; *—*, Wealing at 366 and 405 nm; ↓, Dose insufficient to provoke erythema; ↓ Dose insufficient to provoke wealing; The inaccuracy in the determinations approximately amounts to a factor of 2. Notice the extreme sensitivity regarding the induction of erythema and urticarial swelling which is maximal at 405 nm.

Overdose (6–10 MED) of 257 or 297 nm produced normal late swelling; however 3 MEDs of 366 nm and even 1 MED of 405 nm induced wealing. With 3 MEDs of 436 nm light no wealing could be elicited (higher doses would require more than 1 h irradiation and could therefore not be given). The wealing at 366–405 nm disappeared within 2–3 h without residuum and thus differed from the delayed oedema in EPP.

Microscopy

Skin biopsies were obtained without anaesthesia from the back and the dorsal aspect of the forearm before and 24 h after 3 MED of 405 nm, and from unirradiated skin from thighs and buttocks. Specimens were immediately stored at −80°C.

Skin sections were tested by means of immunofluorescence (IF) procedures (Baart de la Faille-Kuyper, 1969) for IgG, IgA, IgM, IgE, Complement-factors C1q, C4, C3b, C3c, C3d, C5, and human albumin* by the indirect IF technique with monospecific antisera of rabbit origin and a horse anti-rabbit immunoglobulin FITC-labelled conjugate*. Fibrin and related substances (further designated as fibrin) were demonstrated with a FITC-labelled rabbit anti-human fibrinogen conjugate†. The specificity of the above reagents has been mentioned elsewhere (Baart de la Faille-Kuyper & Baart de la Faille, 1974).

The sections were examined with a Zeiss fluorescence and photomicroscope (transmitted light) and a Leitz Orthoplan microscope for incident 'narrow band' illumination (Ploem, 1967).

Mast cells were demonstrated with toluidine blue or by the naphthol AS-D chloro-acetate esterase procedure (Leder, 1964), lipids with 3–4 benzpyrene, oil red and Sudan black.

IF methods showed only normal amounts of the factors tested, but 1 h after 405 nm irradiation, massive fibrin deposits appeared in the superficial dermis (Fig. 2). 24 h later these deposits had practically disappeared. Thrombosis was absent. Mast cells, mostly in and around blood vessel walls (Fig. 3), appeared more numerous than normal; 24h after irradiation degranulation seemed to have occurred.

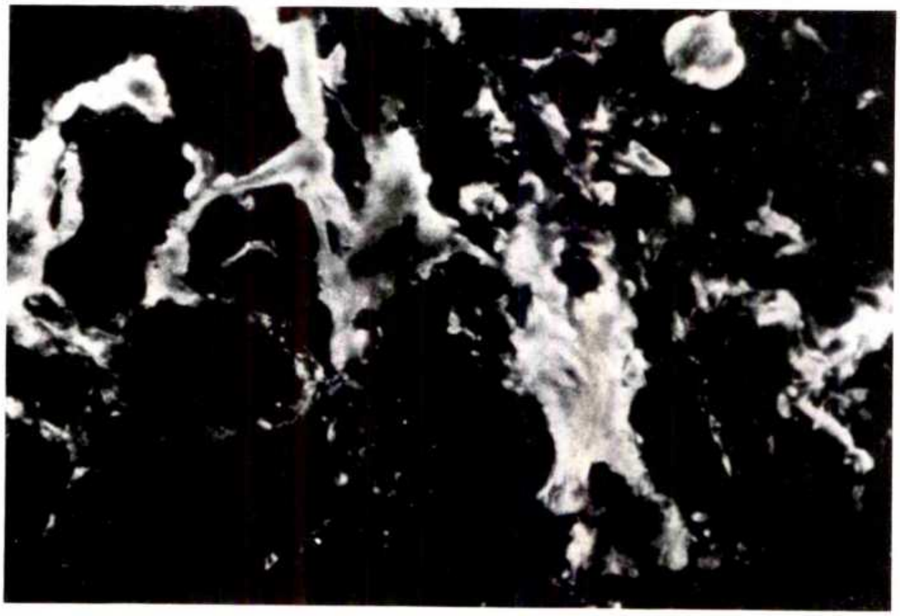

FIGURE 2. Skin of the back, 1 h after irradiation with violet light (405 nm). Direct stain with anti-human fibrin conjugate. Perivascular fibrinous exudate. Leitz Orthoplan microscope (incident illumination) (objective × 54).

Small fat deposits were occasionally observed in epidermal cells and vessel walls 1 h after irradiation.

Miscellaneous investigations

Blood count normal; reticulocytes 0·4%, later 1·8%; haptoglobin, glucose-6-phosphate dehydrogenase, serum iron, iron binding and saturation, methaemoglobin, sulphaemoglobin, blood clotting

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* Central Laboratory, Netherlands Red Cross Blood Transfusion Service, Amsterdam.

† Nordic Pharmaceuticals and Diagnostics, Tilburg.

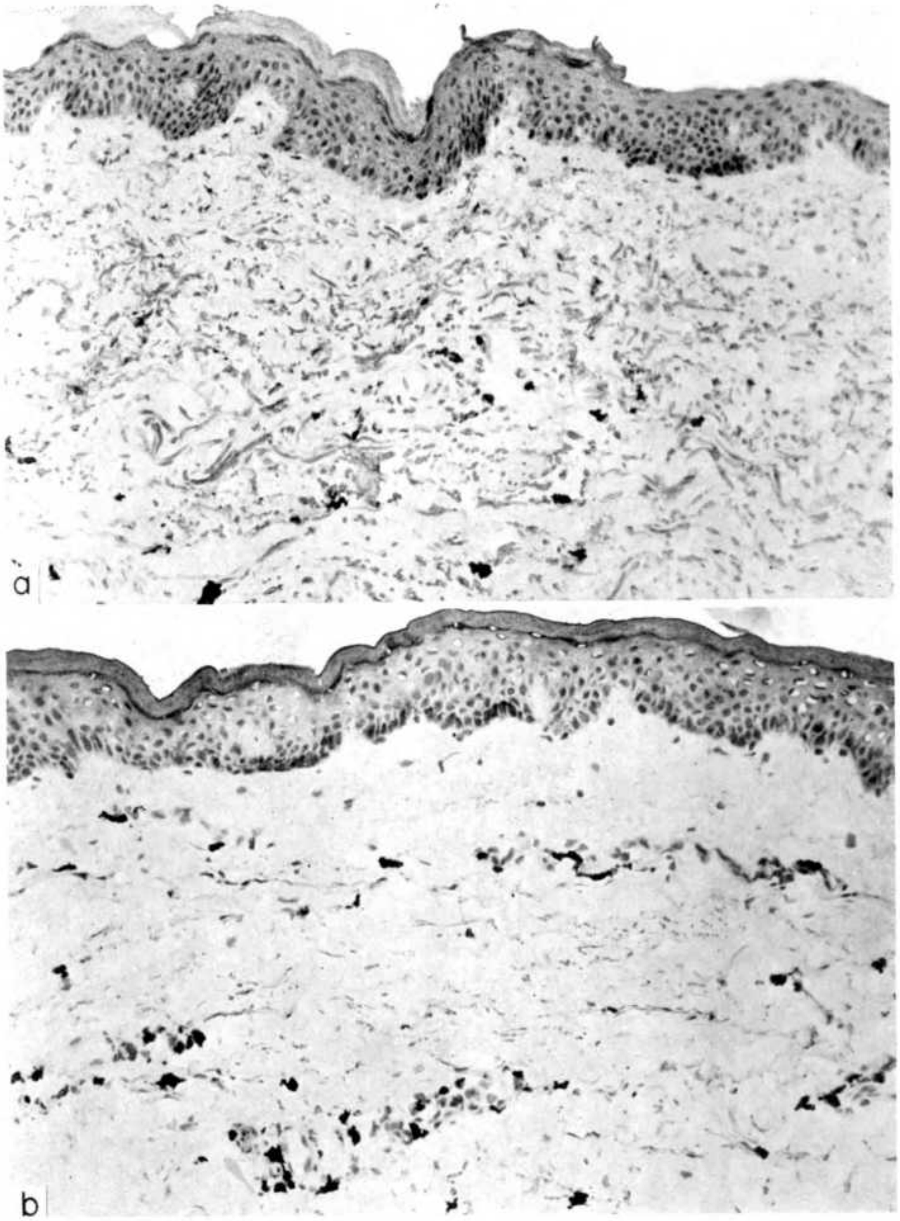

FIGURE 3 a, b. Naphthal ASD chloroacetate technique. Mast cells in the patients skin (b) are present in amounts possibly larger than normal (a) (objective ×25).

serum electrophoresis, SGOT, SGPT, CPK, photo-haemolysis (Schothorst *et al.*, 1970) and Coombs' test, all normal. Faecal occult blood, negative. Plasma Hb 11 mg% (normal <5 mg%); erythrocyte osmotic fragility to various concentrations NaCl decreased; $T_{\frac{1}{2}}\,^{51}$Cr.RBC 20 days (normal 24–36 days); autohaemolysis slightly raised; serum bilirubin 10·4–19·5 mol/l (normal <14); indirect reacting fraction 0·15; LDH 285–400 mol/min/l (normal <200); 3d LDH isoenzyme repeatedly elevated.

Tests for toxoplasmosis, syphilis, malaria, hepatitis (Australia antigen) and ANF–negative. Passive tranfer experiments were not considered justifiable. Red cell, faecal and urinary porphyrins normal. No fluorocytosis.

Bone marrow and liver biopsy: normal, no mastocytosis. ECG, X-rays, of chest, spleen, skull, and extremities—normal.

Therapy

Incidal (Bayer) 3-N-methyl-9-benzyl-tetrahydro-γ-carboline.
The patient's complaints disappeared with Incidal 300 mg daily; he tolerated sunlight much better. This impression appeared to be confirmed by phototests; the wealing dose of 405 nm radiation during treatment increased by over 64 times, whereas the MED (405 nm) was uninfluenced and only showed the customary seasonal variation. The extreme wealing effect of violet light reappeared 3–4 days after stopping therapy (Table 1).

TABLE 1. Threshold doses at 405 nm radiation in log Ws./cm^2 for late erythema and wealing; effect of treatment with Incidal (I)

Date 1971	Treatment	Erythema	Wealing
29 March	Nil	0·6	0·7
21 April	3 days I	1·0	>1·8
27 April	4 days nil	1·2	1·3
28 October	3 days nil after 6 months I	0·7	0·7
12 November	7 days I	0·7	>2·5

> means wealing not provoked with the dose mentioned.

DISCUSSION

A moderate increase in dermal mastocytes seemed present in this case of solar urticaria, but without evident systemic mastocytosis. That this may herald a reticulosis, is a possibility.

Although the hypersensitivity at 405 nm might be suggestive of porphyria (Magnus, 1968), this condition was excluded by normal red cell, faecal and urinary porphyrins and no fluorocytosis. Moreover, the conspicuous, thickened PAS-positive blood vessel walls in light-exposed skin of EPP and porphyria cutanea tarda were not found (Baart de la Faille-Kuyper & Cormane, 1968; Baart de la Faille-Kuyper, 1969). Nor were there the conspicuous fat droplets in epidermal cells and in various dermal structures characteristic of EPP following irradiation with visible light (Baart de la Faille-Kuyper, Rottier & Baart de la Faille, 1968). Evidently hypersensitivity at 405 nm is not specific for porphyria, as witnessed by the findings in our patient.

The urticarial reactions in our patient showed the characteristics of the triple response (Lewis, 1927), a reaction which may be attributable to histamine release. In our case, histamine may possibly be important for initiating the wealing, if the suppressive effect of the antihistamine Incidal is regarded as valid. The fact that Incidal diminishes the wealing dose without changing the minimal erythema dose, points to differences in the pathogenesis of these reactions. The release of histamine might be triggered by direct damage of the mast cells by light. It is unlikely that epidermal mediators play a role in the genesis of the urticarial reactions, because the short period of latency between irradiation and skin reaction does not permit substances to diffuse from epidermis to dermis.

The extensive perivascular deposition of fibrin shortly after irradiation was evidently the result of a short-lived, pathological increase of vascular permeability. As fibrinolysis in solar urticaria was either normal or increased after wealing (Grice *et al.*, 1970) it is understandable that in our case the heavy fibrin deposits had already disappeared 24 hours after irradiation.

According to Ryan, Nishioka & Dawber, (1971) perivascular fibrin deposition is not a feature of histamine activity. In this respect, other mediators may play a part. A definite role of kinins seemed unlikely for the following reasons: the skin reactions were not painful, they were of short duration and could be counteracted by an antihistamine. Complement factors seemed excluded as mediators, because C1q, C4, C3b, C3c, C3d and C5 were not demonstrable. It might be considered that circulating factors have a role from the results of passive transfer experiments and the fact that circulatory occlusion during irradiation may prevent or delay a weal developing (Grice *et al.*, 1970). However, without an intact circulation, wealing cannot occur and so mere delay of wealing until the circulation is restored is inconclusive. The gradual depletion of such a factor as a result of prolonged light exposure might account for the enhanced tolerance to light in our patient during a holiday period. However, the possibility of a local tachyphylaxis should also be considered.

In our opinion it is not justified to deduce from the results of passive and reverse passive transfer experiments in solar urticaria that the mechanism of the pathological reactions is allergic or auto-allergic. The absence of detectable quantities of immunoglobulins and complement factors at the site of pathology does not favour an antibody-mediated pathogenesis of the condition.

We assume that the puzzling haemolysis (possibly of temporary character) as well as the increased levels of various LDH serum factors are a coincidence and do not bear a relationship to the skin condition. The decreased red cell survival might be related to a combination of a slight defect in the cell membrane and/or a slightly impaired production of haemoglobin of some of the red cells.

ACKNOWLEDGMENT

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Coeliac disease with primary cutaneous amyloidosis

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SUMMARY

A case is described where the patient suffered from coeliac disease, and a widespread eczema which was succeeded by lichen amyloidosus.

The occurrence of skin lesions in patients with coeliac disease has been well recognized for some years. Bennett, Hunter & Vaughan (1932) reported fifteen cases of idiopathic steatorrhoea, seven of whom had skin lesions. Since then there have been many reports. In particular, Cooke, Peeney & Hawkins (1953) found twenty patients of their series of 100 cases who had skin lesions, and Badenoch (1960) reported 163 cases, of whom fifteen had eczema and three had presented with their skin eruption. The skin changes occurring in malabsorption states have been reviewed by Wells (1962), who emphasized that they are not specific in their clinical appearance.

Many patients have become pigmented, while others have developed eczema of differing patterns. Usually the changes have been of a patchy or discoid distribution with hyperpigmentation particularly at the edge of a lesion.

Lichenification has been seen frequently, and erythroderma has occurred in a few. In some patients the changes have apparently been those of psoriasis and the eruption has fluctuated with the activity of the steatorrhoea. Often the skin has cleared rapidly after treatment with a gluten-free diet has been started. Some of the skin changes have been attributed to hypocalcaemia as described by Dent & Garretts (1960), but many patients have had normal serum calcium levels.

The patient reported here presented with eczema before discovery of his coeliac disease. Since taking a gluten-free diet, his eczema has improved but the skin changes of lichen amyloidosus have become apparent. The association of coeliac disease with lichen amyloidosus does not seem to have been recorded before.

CASE REPORT

A 61-year-old man presented with an 8-week history of a rash on the hands. It had started as acute, vesicular eczema of the palms, which had become dry and scaling by the time he was first seen. During the next 6 months this continued and lichenification of the calves was noted. At the end of this period he had a further flare-up with acute, vesicular eczema of the fingers and nail folds. His shins and thighs showed dry scaling and lichenification, confirmed by skin biopsy. Patch tests with a large number of potential allergens were negative. It was at this time that he gave a history that soon after the onset of his skin trouble he had started to have explosive diarrhoea 2–4 times daily, the stools being pale and bulky. He was thought to have steatorrhoea, and at that time his full blood count was normal, with a serum folate of 0·6 μmg/ml, and a faecal fat excretion of 34·4 g/day. He was investigated further by Dr B.Creamer at St Thomas' Hospital, who carried out a jejunal biopsy, which showed a flat mucosa typical of coeliac disease.

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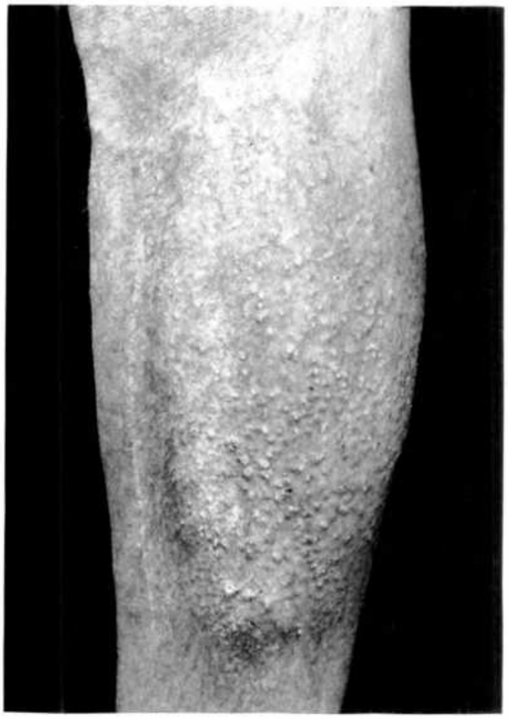

FIGURE 1. Typical appearance of lichen amyloidosus on shin.

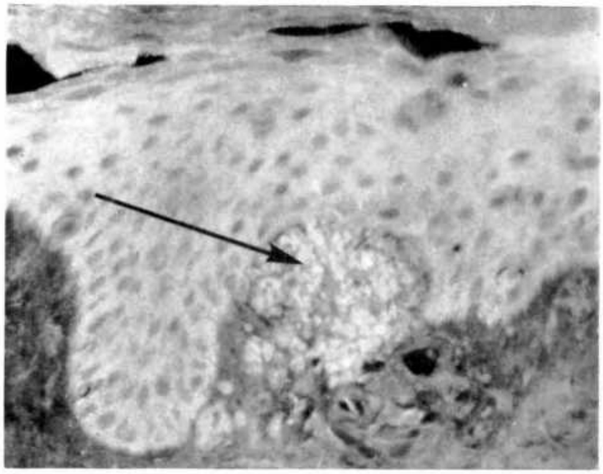

FIGURE 2. Deposition of amyloid (arrowed) in dermal papilla (Thioflavine—T, × 200).

He was started on a gluten-free diet and his diarrhoea stopped at once. His skin gradually improved so that over the next year the hand eczema cleared, and he was left with apparent lichenification of the calves. This was seen to be made up of discrete, brown, firm, dome-shaped, rough and excoriated papules of the type seen in lichen amyloidosus (Fig. 1). A biopsy showed the presence of amyloid in some of the dermal papillae, using congo red, methyl violet and thioflavine-T stains (Fig. 2). This confirmed the diagnosis of lichen amyloidosus. Further sections taken from his original skin biopsy were also stained in this way and deposits of amyloid were found to be present. Biopsies were then taken from an abnormal area and from uninvolved skin on the thigh and snap-frozen in liquid nitrogen. Examination by direct immunofluorescence for deposition of immunoglobulins, complement and fibrin was negative.

At present his full blood count is normal; ESR 2 mm/h; total serum protein 7·4 g/100 ml (albumin 4·2 g/100 ml, globulin 3·2 g/100 ml) with a normal electrophoretic pattern; serum calcium 9·2 mg/100 m l, phosphate 2·6 mg/100 ml.

The patient remains very well but the pruritic areas of lichen amyloidosus have not been changed by topical treatment.

DISCUSSION

Three clinical patterns of primary cutaneous amyloidosis are recognized. The most common is the lichenoid papular type of which this patient is an example. The other patterns are macular amyloidosis, seen as a rippled pigmentation, and a rarer localized tumefactive lesion. Investigation of each group has failed to reveal any consistent underlying biochemical abnormality, and, in particular, no suggestion of myelomatosis or other systemic disease has been found (Tay & Dacosta, 1970; Brownstein & Helwig, 1970; Black & Wilson Jones, 1971). This is important since primary systemic amyloidosis may present as malabsorption syndrome. There is nothing to suggest that this patient has systemic amyloidosis, the skin changes of which tend to be less uniform and are often purpuric, the amyloid deposits involving blood vessels, and extend more diffusely through the dermis.

It has been suggested that the deposition of amyloid may be secondary to lichenification, but amyloid may be found in clinically normal skin in patients with lichen amyloidosus with no overlying change in the epidermis (Brownstein & Helwig, 1970). No acceptable explanation has been found yet, but Black & Wilson Jones (1971) felt that, as there is apparent damage to the basal layer of the epidermis in this condition, the degenerating epidermal cells may be converted into amyloid. There is no obvious cause for the concurrence of the skin changes and coeliac disease in this man.

It is well-known that dermatitis herpetiformis is associated with changes in the small intestine that are indistinguishable from those of coeliac disease (Marks, Shuster & Watson, 1966). The skin changes in this patient have never suggested dermatitis herpetiformis, clinically or histologically. Furthermore, direct immunofluorescence studies on involved and normal skin in dermatitis herpetiformis would be expected to show deposition of IgA in the dermal papillae (Seah *et al.*, 1972). These studies were negative in this man.

There seems to be no report of a similar combination of lichen amyloidosus and coeliac syndrome. However, there have been many reports of pigmentation and lichenification in the past, and it is possible that retrospective study of the histology might show the deposition of amyloid in some patients.

ACKNOWLEDGMENTS

We are grateful to Dr G.C.Wells for his permission to publish this case under his care, and for his help and advice, and to the Department of Medical Illustration, St John's Hospital for Diseases of the Skin.

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Correspondence

PIEZOGENIC PEDAL PAPULES

SIR, Harman & Matthews (1974) report a case of painful piezogenic pedal papules and present a histological photomicrograph showing a nodule of fat protruding into the dermis. They noted no fat necrosis, fibrous or vascular changes, and stated that their presentation appeared to be the first histological vindication of the theory of Shelley & Rawnsley (1968).

Schlappner *et al.* (1972), however, have previously presented a detailed histological study of both painful and non-painful piezogenic pedal papules. They found that in two patients with painful papules the normal anatomical relationships of fat, fibrous tissue and skin were disturbed. The painful papules exhibited (1) thickening of the dermis with a few irregular islands of adipose cells, (2) loss of compartmentalization of the small fat lobules in the deeper dermis and at the dermal-subcutaneous junction, and (3) loss or thinning of the trabeculae of the subcutaneous tissue. They noted that on gross sectioning there were circular holes in the lower portion of the dermis through which subcutaneous fat could be made to protrude on pressure. Histological sections showed a globule of fatty tissue protruding into the lower dermis. A detailed pathological description summarizing the findings in ten cases of non-painful pedal papules was also presented.

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PSEUDO-MONILETHRIX

SIR, Our report on this previously undescribed hair anomaly was published in 1973 (Bentley-Phillips & Bayles, 1973) showing how it differed from classical monilethrix and demonstrating its frequent association with a curious form of pili torti and a forme fruste of trichorrhexis nodosa. Pseudo-monilethrix, studied in a very large family of white South Africans (seventy-six members), was inherited as an autosomal dominant trait with variable penetrance (Family A). We have since investigated three further families, B, C and D respectively of Indian, British and German origin (Fig. 1.) and the same genetic pattern has emerged (Bentley-Phillips, Bayles & Grace, 1974).

Recently we have encountered an even more interesting Indian family of forty-five members (Family E) where pseudo-monilethrix has been transmitted from both sides of the family, the proband's materna. and paternal grandmothers being affected and with virtually 100% of the kindred showing hair anomaliesl There are thirty-four cases of pseudo-monilethrix, fourteen of pili torti and nine of trichorrhexis nodosa. Double pathology is seen in eleven cases and triple pathology in two (Fig. 2).

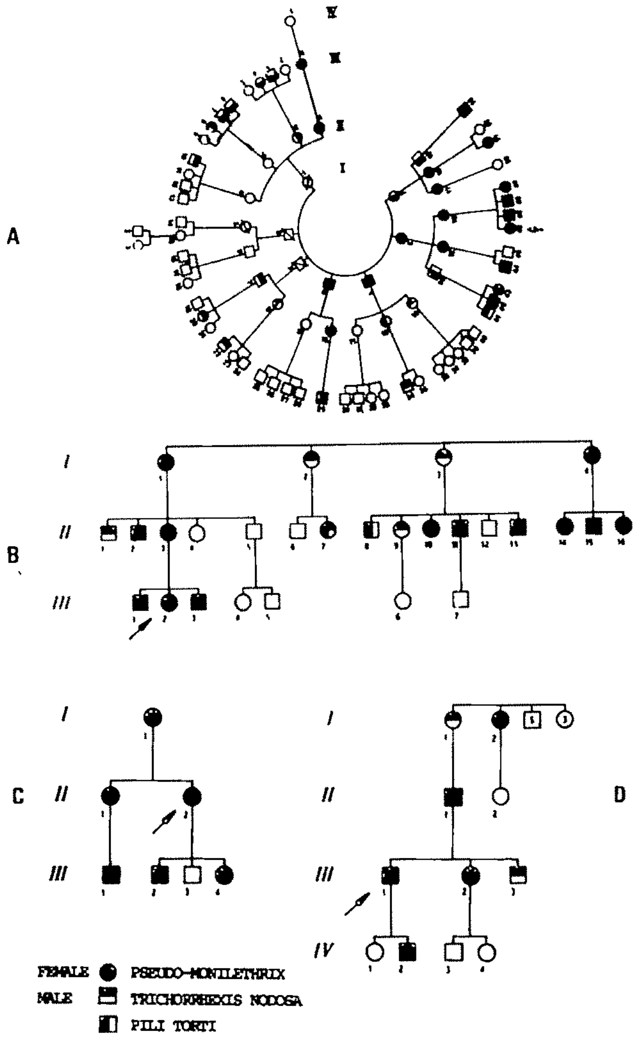

FIGURE 1. Pedigrees of Families A, B, C and D.

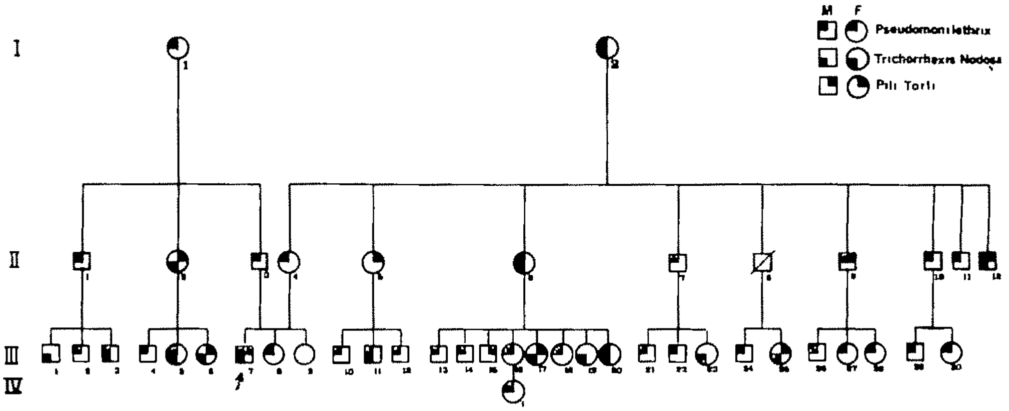

FIGURE 2. Pedigree of Family E.

Since identical clinical and genetic patterns have been established in five families of different racial backgrounds, it would appear that this syndrome deserves a place among the already familiar hair disorders.

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Thesis

Measurements of the surface of the skin. Thesis presented by F.A.J.Thiele 1973, Nijmegen, Holland. Pp. 140.

This thesis describes some of the author's experiments carried out at the Department of Dermatology at Nijmegen, and at Unilever Research Laboratories, Vlaardingen, over the past decade. The subjects covered are diverse—insensible water loss, sweating and CO_2 production—but are brought together as delicate techniques for assessing skin damage caused by external agents. This is a compilation of mainly published work with additional introductory remarks and calculations.

The first papers relate to water loss with the conclusion that the driving force for transepidermal water loss comes the subepidermal capillaries.

A short paper amplifies the hypothesis that the sweat gland may be comparable to a heat pipe—evaporation of water at the base of the duct, condensation at the upper part of the duct and return through the mucous layer allowing rapid transfer of heat to the surface without loss of fluid from the surface.

The techniques for measuring CO_2 release from small areas of skin (as already published in this Journal, **89**, 373 and 565) are described in detail, and in the final paper are used to study skin damaged by external agents. It is also shown that activity of the sweat gland itself is modified by external factors.

Book Reviews

Multiple Choice Questions in Dermatology. Compiled by S.K.Goolamali (1974), Edinburgh and London: Churchill Livingstone. Pp. 91 Price £1.00.

Multiple choice questions have become such an integral part of both qualifying and higher examinations that the essay type of question is fast disappearing. In his preface Dr Goolamali points out that a multiple choice book concerned with dermatology has not previously appeared. The book is divided into ten papers consisting of twenty questions each. Dr Goolamali has rightly concentrated on those aspects of dermatology which relate to internal medicine. Whilst agreeing that these questions will be of value to M.R.C.P. candidates or graduates preparing for the London Dermatology Diploma, I feel that many of them are too 'hard' for final year medical students. Furthermore, I believe that the value of the book would have been enhanced if one or two pertinent references could have been supplied alongside many of the individual questions.

Martin M.Black

Hair Transplant Surgery. O.T.Norwood (1974) Illinois: Charles C.Thomas. Price £4.28.

This short monograph describes clearly the technique of skin punch transplantation as a cosmetic procedure for the treatment of male pattern baldness. Any dermatologist who wishes to start using this technique would obtain useful information from the detailed, pleasantly illustrated text.

This method of treating a physiological process has not been accepted by British Dermatologists, primarily because of its purely cosmetic aspect in a group of patients who are on the fringe of those requiring psychiatric help because of their pathological or obsessional concern about their appearance. Many of those patients treated by the method in the United States belong to this group of individuals, and not a few have been exploited financially by their cosmetic surgeons. While the text as presented is acceptable, the principle of hair transplant surgery is not.

D.D.Munro

Lehrbuch der Haut Krankheiten und Venerischen Infectionen fur Studierende und Ärzte. THEODOR NASEMANN AND WOLFARD SAUERBREY (1974) Berlin: Springer Verlag. Pp. 400. Price £7.86.

In this volume of some 400 pages, there are approximately fifty pages devoted to venereology. The text is in double column, easy to read and well divided with headings and sub-headings. There is a profusion of tables, schematic drawings, photo-micrographs and clinical pictures. The photographs tend to be larger than life, and many examples of tumours and the pictures of lupus vulgaris are so advanced as to be caricatures of the full blown disease, rather than the more mild and early forms, which are becoming more familiar.

There is no sparing of detail, and one is delighted to see that continental students in Germany are not presented with watered-down versions 'for students and general practitioners', but instead here is a working manual of Modern Dermatology beautifully presented, which would satisfy many consultant dermatologists.

Lyell's disease, Sneddon–Wilkinson Disease, and references to Pustular Psoriasis of the Barber Type are all here. The British Dermatologist who acquires this volume will be well pleased with the contents, and the views expressed are familiar and acceptable. Viral disorders are especially well dealt with and Professor Nasemann is of course an expert in this field.

For Teachers of Dermatology, the classification of aetiology in skin diseases, and the schematic drawings could well be adopted as refreshing modes for teaching for our own students. The authors are to be congratulated in fully achieving their aim.

MAURICE GARRETTS

Funktionelle Dermatologie. GÜNTHER STUTTGEN AND HANS SCHAEFER (1974) Berlin: Springer. Pp. 531. Price £32.46.

This volume is an up to date encyclopaedia of modern scientific knowledge about the skin.

There are chapters on Morphology of the Epidermis, Development of Skin, The Corium, Mucous Membranes, Metabolic Upsets of the Skin and so on. Nails, Hair, Sweat Glands are then dealt with, and thereafter follow chapters on the Biochemistry of Sebum.

All these subjects are written as review articles with current references. The Pharmacological basis of Dermatological Therapy is a large contribution with some 170 references.

Functional Dermatology for the Authors is the outcome of Biochemistry, Physiology, Morphology and Therapy in skin disease. It is a useful concept and the multi-discipline approach is pursued throughout the work. Many of the references are German, and much of the work is original.

There are over 300 tables of factual information, with the references from which they have been extracted. Research workers would find this a mine of information with regard to basic values in normal and certain abnormal skin tissues. Towards the end of the work is a Diagnostic Index with a few lines on each of the disorders mentioned, and references to the text where various facets, (morphological, biochemical, therapeutic etc.) are discussed.

For readers of German Dermatological literature, there is a chapter on the Nature of Official German Topical Bases or Vehicles, and commonly used medicaments.

Wiener was responsible for bringing together a great deal of General Medical and Dermatological knowledge. Rothman approached this type of knowledge in a more basic and scientific manner. Stüttgen and Schaeffer have taken their material from the literature of the last 20 years, and no Germanr-eading dermatologist could afford to be without this volume.

MAURICE GARRETTS

Common Skin Diseases of children in the tropics and **Skin Diseases in children in temperate climates.** ALAN B.SHRANK. Colour slides reproduced by 'Teaching Aids at Low Cost' for Institute of Child Health, London W.C.1, and Medical Recording Service Foundation, Chelmsford CM1 3EH. Price from 90p unmounted to £6.00 in folder with cassette, plus VAT.

These two sets of twenty-four colour transparencies (2 × 2 inch) have been assembled by Dr Shrank and reproduced for the Institute of Child Health by TALC (Teaching Aids at Low Cost). Each set is accompanied by four or seven pages of text, giving a brief description of the slides, and asking a few pertinent questions. Alternatively, a lecture on a cassette is available.

These slides are aimed especially at health workers, including medical personnel, in developing countries, but are also suitable for nurses, medical students and even doctors in this country. The selection of subjects for the slides is sensible, with an emphasis towards infective conditions. The quality of the transparencies is mainly good, but some have inevitably lost their correct colour values in the reproduction, and two or three of the diseases in temperate climates were not recognized when projected before a group of Dermatologists.

The cost of 90 pence unmounted or £1.30 mounted (even cheaper in developing countries) does indeed make this a teaching aid at low cost which deserves to succeed. There must be many audiences which would benefit from lecture demonstrations of this type, although for the solitary viewer the colour atlases, which cover somewhat similar ground, might be preferred.

R.H.CHAMPION

Microbiology of the Human Skin. W.C.NOBLE and DOROTHY A.SOMERVILLE (1974) London: W.B.Saunders Company Ltd. Pp. 341. Price £1.00.

During the last decade there has been a tremendous increase in interest, shared by clinicians and microbiologists, in the distribution and composition of the microbial communities inhabiting the human skin. New and sophisticated methods of studying this biocenose have been introduced, and an immense amount of information relating to cutaneous ecology has been published.

The authors of this excellent book have themselves made distinguished contributions to this knowledge, and, as readers will realize, show an enviable gift in summarizing the results obtained by other investigators in the field. Tables, in which the results obtained in different studies are compared, are used throughout the book, and these are extremely valuable. All sections are well illustrated and each chapter has an extensive and up to date list of references.

The book is divided into three sections. The first describes the attributes of the skin which characterize it as a habitat for living organisms. An interesting chapter on the density, distribution and transmission of microbial populations completes this section. This chapter is well illustrated with tables and histograms. The reasons for the conflicting results obtained by different investigators are discussed. The available information on the interactions of micro-organisms with the substrate and with each other is well summarized.

The second section describes in turn the different groups of bacteria, fungi and viruses living harmlessly or as pathogens on the skin. Most attention is given to the dominant members of the normal flora, but reference is made to the rarer cutaneous pathogens. One rather surprising arrangement is the inclusion of *Corynebacterium diphtheriae* and a discussion of cutaneous diphtheria in the general chapter on the corynebacteria, instead of in the following one which is entitled 'Corynebacteria as pathogens'.

In the chapters concerned with the Micrococcaceae taxonomic problems are clearly presented. The many studies of skin carriage of *Staphylococcus aureus*, and the distribution and potential pathogenicity of coagulase-negative strains are well summarized. The section ends with a chapter on the skin fauna which does seem rather scanty. It deals briefly with *Demodex* and *Sarcoptes scabiei* and lists certain pathogens but omits all reference to the human lice, which are once more becoming a public health problem.

The third section is entitled 'Ecology of the skin'. It includes a very valuable and interesting chapter on the microbiology of non-infective pathological skin conditions. There is a chapter on the flora of special skin sites in which there is some repetition of information supplied in section (1). Another chapter describes current knowledge of the effects of host factors and external agents on the density and composition of cutaneous communities. The final chapter describes the various methods used for sampling the cutaneous flora, and in an appendix the actual results obtained from sampling the skin of five individuals are supplied.

One possible criticism of this book is the somewhat detached view taken by the authors. They are careful not to draw conclusions from the studies they have summarized. The sections would, however, make more exciting reading if the authors were on occasion a little more dogmatic, since positive attitudes are the best stimulants to further research. This is only a minor criticism. The book is an excellent digest of our knowledge of cutaneous microbiology, and a must for all clinicians and microbiologists investigating the ecology of the normal and pathological human skin.

MARY J.MARPLES

Xanthoma formation and other tissue reactions to Hyperlipidemias. O.BRAUN-FALCO, C.KELLER and N.ZOLLNER (1973) Basel: Karger. Pp. 153. Price £5.10.

In his introduction Braun-Falco points out that the purpose of the symposium was to study the problems of basic research. The eighteen communications of which the Proceedings are composed, all given by well-known research workers in this field, achieve that purpose admirably.

The present state of knowledge concerning lipoproteins in general is dealt with first. Thereafter the vascular lesion is considered with regard to the similarity of its lipoproteins to those in the serum and to the mechanism whereby these lipoproteins penetrate the endothelial lining and are then selectively retained in the blood vessel wall. Similar studies are described in relation to the cutaneous lesion, together with a description of the varieties of xanthomata which are to be found in the five types of hyperlipoproteinaemia.

There are three clinical papers on rare conditions which illustrate various aspects of hyperlipidaemia. Early diagnosis is described by analyzing cord blood and finally therapy is excellently and critically dealt with by considering when to treat, what drugs to use and how to assess the results.

Excellently illustrated and printed on good paper, this publication is to be highly recommended to anyone concerned with this field of study. Only two criticisms are to be made. First, the fact that the Proceedings appear 2 years after the symposium. That this is not necessary is shown by the fact that the Proceedings of the series of Symposia on Advanced Medicine held at the Royal College of Physicians are published within a few months. Secondly, there is no index.

P.BORRIE

British Journal of Dermatology (1975) **92**, 120.

News and Notices

Announcement

The Annual Meeting of the British Association of Dermatologists will take place in Cambridge on 3–5 July 1975, under the presidency of Dr Arthur Rook. As an innovation, there will be additional papers of a laboratory orientated nature on the Friday afternoon for those who do not wish to join in the social and sporting activities. The rest of the programme will be as usual. Papers are invited and should be sent in the form of a summary of approximately 200 words (6 copies) to the Hon. Secretary, Dr J.S.Pegum, The London Hospital, London E.1.1BB, before 1 March 1975. If there is more than one author, please underline the name of the speaker. The British Journal of Dermatology hopes to publish, in a supplement, the summary of these papers which are accepted. These, therefore, should be detailed, precise and explicit and avoid such phrases as 'the results will be discussed'. Any references should be set out as required by the British Journal of Dermatology.

The Second Annual Hair Transplant Symposium and Workshop

The American Society for Dermatologic Surgery and The American Academy of Facial Plastic and Reconstructive Surgery, Inc., are co-sponsoring a conference on hair transplantation, to be held on 14 and 15 February 1975, at the Stough Dermatology and Cutaneous Surgery Clinic, P.A., Doctors Park, Hot Springs, Arkansas 71901. For further information, contact: Dr D.B.Stough, III, Program Director. (Address as above.)

New Journal: 'CONTACT DERMATITIS'

A new Journal entitled 'CONTACT DERMATITIS: Environmental and Occupational Dermatitis', will be published quarterly from January 1975. The Editor, as of the Newsletter of the International Contact Dermatitis Research Group, from which the new Journal has grown, is Dr C.D.Calnan, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ. The publishers are Munksgaard, Post Box 2148, 35 Nørre Søgade, DK-1016, Copenhagen K, Denmark.

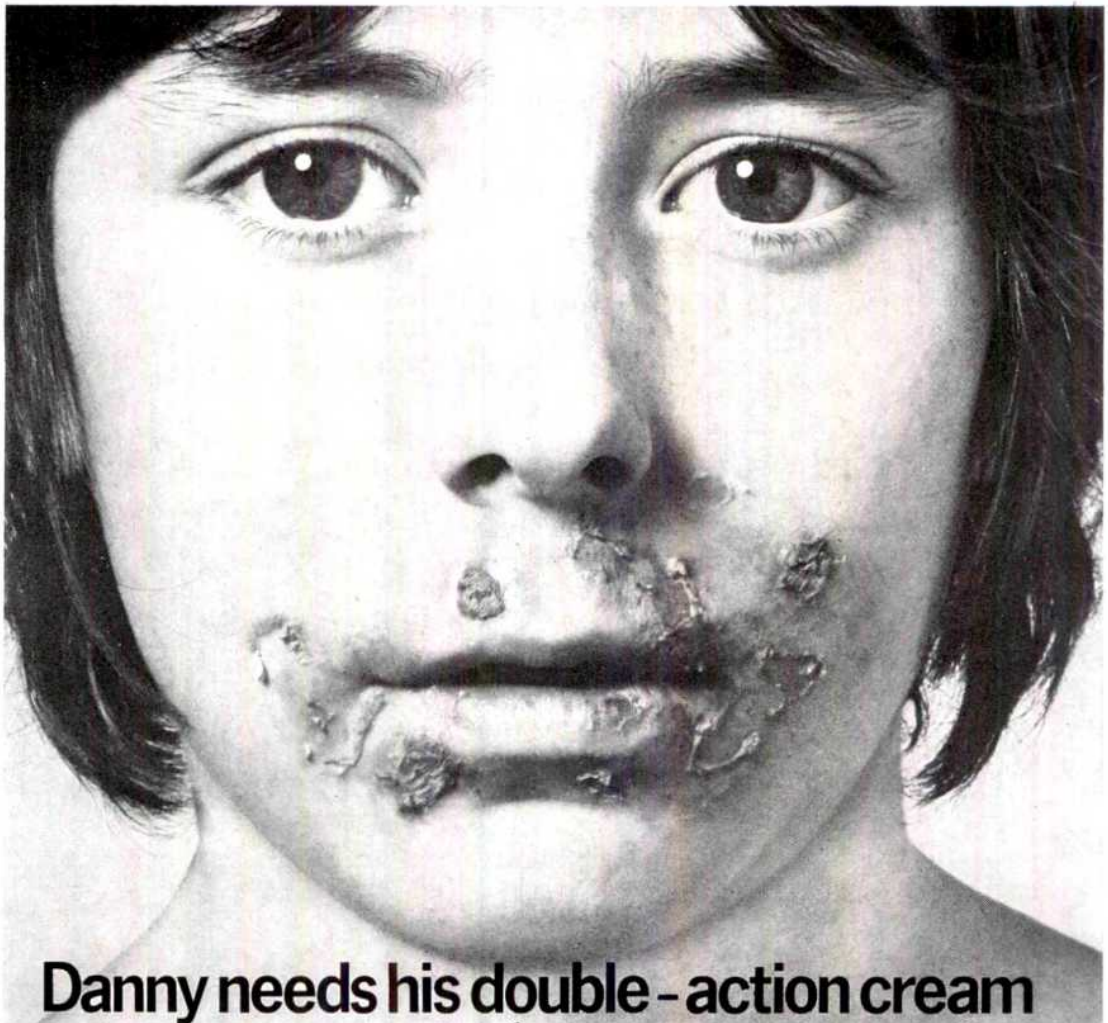
APPOINTMENTS

Institute of Dermatology, London

Dr E.Wilson Jones has been appointed, from the 1 October 1974, to a Personal Chair in Dermatopathology at the Institute of Dermatology, St John's Hospital for Diseases of the Skin, London, where he has been Reader for the past 5 years, and Honorary Consultant for the past 10 years. His interests and numerous publications cover all aspects of histopathology of the skin.

Royal Infirmary of Edinburgh

Dr John Angus Alexander Hunter has been appointed Consultant Dermatologist to the Lothian Health Board, Scotland, with duties in the Royal Infirmary of Edinburgh. Dr Hunter qualified M.B.ChB. Edinburgh in 1963 and became M.R.C.P.E. with Dermatology as a special subject in 1967. His training in Dermatology took place in the Royal Infirmary, Edinburgh, The Institute of Dermatology, London, and as an Exchange Research Fellow for a year at the University of Minnesota, U.S.A., working with Dr A.S.Zelickson. His research interests are mainly concerned with Electron Microscopy of the skin and he has published several papers on this subject.



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Clinical and Experimental Pharmacology and Physiology

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Volume 1, Number 5, September–October 1974

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Y. Lamboeuf, G. de Saint-Blanquat and R. Derache

Properties of the cell membrane of developing skeletal muscle fibres in culture and its sensitivity to acetylcholine
William F. Dryden, Solomon D. Erulkar and Gabriel de la Haba

Inhibition of renin-angiotensin induced drinking in the cat by enzyme inhibitors and by analogue antagonists of angiotensin II
M.J. Cooling and M.D. Day

Disposition of serotonin in the rat blood vessels and heart
B.A. Berkowitz, C.-H. Lee and S. Spector

Actions of the sympathomimetic bronchodilator, trimetoquinol (AQL 208) on the cardiac, respiratory and skeletal muscle systems in the anaesthetized cat, and on cat isolated atrial and tracheal preparations
J. Houston and I.W. Rodger

Interaction of some drugs, metal ions, and alcohols with rat liver microsomes as studied with a fluorescent probe
Donald J. Birkett

Effects of tricyclic antidepressants on uptake and release of ^{3}H-noradrenaline in isolated guinea-pig atria
D.F. Story, M.E. Story, M.W. McCulloch, W. Hope and M.J. Rand

Exercise tolerance after anti-anginal drugs: a controlled trial of sustained-release preparations of glycerol trinitrate and alprenolol
O. Momrak Haugan, G. Nyeberg and E-M. Lindseth Ditlefsen

Regional variation in the response of the rat vas deferens to field stimulation, to noradrenaline and to tyramine
Jocelyn N. Pennefather, Ludmila Vardolov and Penelope Heath

On the role of serotonin in the pathogenesis of pulmonary hypertension induced by anorectic drugs, an experimental study in the isolated, perfused rat lung. I. Aminorex, chlorphentermine and phenmetrazine
K.-U. Seiler, Gerda Tamm and O. Wassermann

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Journal of the Society of

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VOL. 25 NO. 7 JULY 1974

J. A. SWIFT AND B. BEWS. The chemistry of human hair cuticle—II: The isolation and amino acid analysis of the cell membranes and A-layer.

T. J. ELLIOT. Use of a laboratory model to evaluate the factors influencing the performance of depilatories.

MEDAL LECTURE

F. J. EBLING. Sex hormones and skin.

The *Journal of the Society of Cosmetic Chemists* is published thirteen times a year at £21.00 ($50.40) per annum, post free. New subscriptions and requests for specimen copies of the journal should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL.

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The purpose of this new journal is to provide a medium for the rapid publication of research papers and review articles of high quality in the field of immunogenetics. Its scope includes the immunogenetics of antigens, such as proteins, histocompatibility and blood group antigens; of antibodies as well as the ontogenetic development and the evolution of immunoglobulins; the immune response and the complement system. Other topics will include the genetics of tolerance-induction and of membrane antigens of the immunocyte.

Volume 1, Number 4, August 1974

W. H. Price, Irene M. Newlands and Thomas Ferguson. Immunoglobulin levels in subjects with sex chromosome abnormalities

Ken Furakawa, Hisao Takizawa and Shoei Iseki. A family case of blood group B variants

Alena Lengerová, Jana Pěknicová and Zora Pokorná. Structural relationships between individual H-2 specificities on the cell surface

J. H. Edwards. HL-A and disease. The detection of associations

C. H. J. Ford and M. W. Elves. The production of cytotoxic antileucocyte antibodies by parous sheep

Nobuhiko Tada, Katsuaki Itakura and Miki Aizawa. Genetic control of the antibody response in inbred rats

Heinz W. Kunz, Thomas J. Gill III and Barbara Borland. The genetic linkage of the immune response to poly(Glu^{52}Lys^{33}Tyr^{15}) to the major histocompatibility locus in inbred rats

Heinz W. Kunz and Thomas J. Gill, III. The effects of antigenic isomerism and antigenic aggregation on the antibody response in inbred rats.

B. Cinader. Six years of the International Union of Immunological Societies.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
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British Journal of Dermatology, Volume 92, Number 1, January 1975

CONTENTS

PAGE

Clinical and Laboratory Investigations

Electrical Potential Difference between Basal Cell Carcinoma, Benign Inflammatory Lesions and Normal Tissue 1
R. E. WOODROUGH, G. CANTI AND B. W. WATSON
Lymphocyte Transformation Test in Allergic Contact Nickel Dermatitis 9
J. M. GIMENEZ-CAMARASA, P. GARCIA-CALDERON, J. ASENSIO AND J. M. DE MORAGAS
Atopic Disease and Serum Immunoglobulin-E 17
HENRY E. JONES, JOYCE C. INOUYE, JOSEPH L. MCGERITY AND CHARLES W. LEWIS
The Interaction of Concanavalin A and the Surface Coat of Stratified Squamous Epithelium 27
J. M. J. VAN LIS AND G. L. KALSBEEK
Antireticulin Antibodies in Dermatitis Herpetiformis and Adult Coeliac Disease—Their Relationship to a Gluten Free Diet and Jejunal Histology 37
M. LANCASTER-SMITH, PARVEEN KUMAR, M. L. CLARK, R. MARKS AND G. D. JOHNSON
Plasma β-MSH Levels in Acne Vulgaris 43
A. J. THODY, S. K. GOOLAMALI, J. L. BURTON, N. A. PLUMMER AND S. SHUSTER
The Erythemal Action Spectrum and Deoxyribonucleic Acid Repair Synthesis in Xeroderma Pigmentosum 49
C. A. RAMSAY AND F. GIANNELLI
The Influence of Treatment of the Paralesional Skin with Dithranol on Healing in Psoriasis 57
L. STANKLER AND VINAY K. DAVE
Microvascular Response to Local Application of Dequalinium Chloride—A Vital Microscopical Study of Hamster's Cheek Pouch and a Microangiographic Study of Rabbit's Ear 63
HÅKAN MOBACKEN AND MIKAEL ROMANUS
Plasma Vitamin A Levels in the Dyskeratoses 73
P. D. MIER, J. VAN DEN HURK AND E. VAN ROSSUM
The Bacteriological Eco-system of the Skin of Children in an African Tropical Environment (Tanzania) 77
HERBERT NSANZUMUHIRE, AARON JOHN E. MASAWE AND FREDERICK S. MHALU

Pharmacology and Treatment

Treatment of Solar Keratoses with a 5-Fluorouracil and Salicylic Acid Varnish 85
J. C. ALMEIDA GONCALVES
5-Fluorouracil in the Treatment of Common Warts of the Hands—a Double-Blind Study 89
J. C. ALMEIDA GONCALVES
A Controlled Trial on the Use of Topical 5-Fluorouracil on Viral Warts 93
M. W. HURSTHOUSE
Anti-viral Treatment of Warts 97
WARWICK L. MORISON

Case Reports

Solar Urticaria—a Case with Possible Increase of Skin Mast Cells 101
H. BAART DE LA FAILLE, P. B. ROTTIER AND E. H. BAART DE LA FAILLE-KUYPER
Coeliac Disease with Primary Cutaneous Amyloidosis 109
D. G. C. PRESBURY AND W. A. D. GRIFFITHS

Correspondence

Piezogenic Pedal Papules 113
ROBERT I. RUDOLPH
Pseudo-Monilethrix 113
B. BENTLEY-PHILLIPS AND MARGARET A. H. BAYLES

Thesis and Book Reviews 116

News and Notices 120

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1. Brit. J. Derm (in press)
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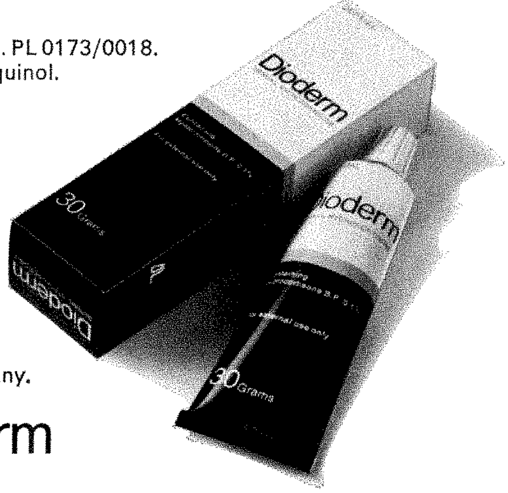

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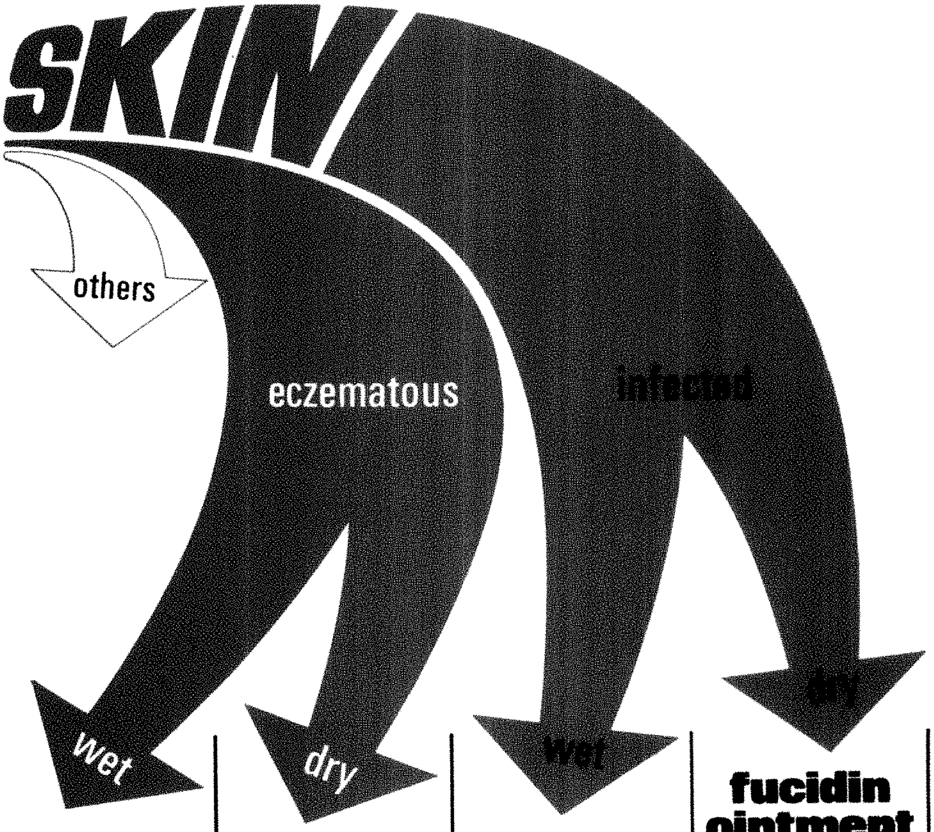

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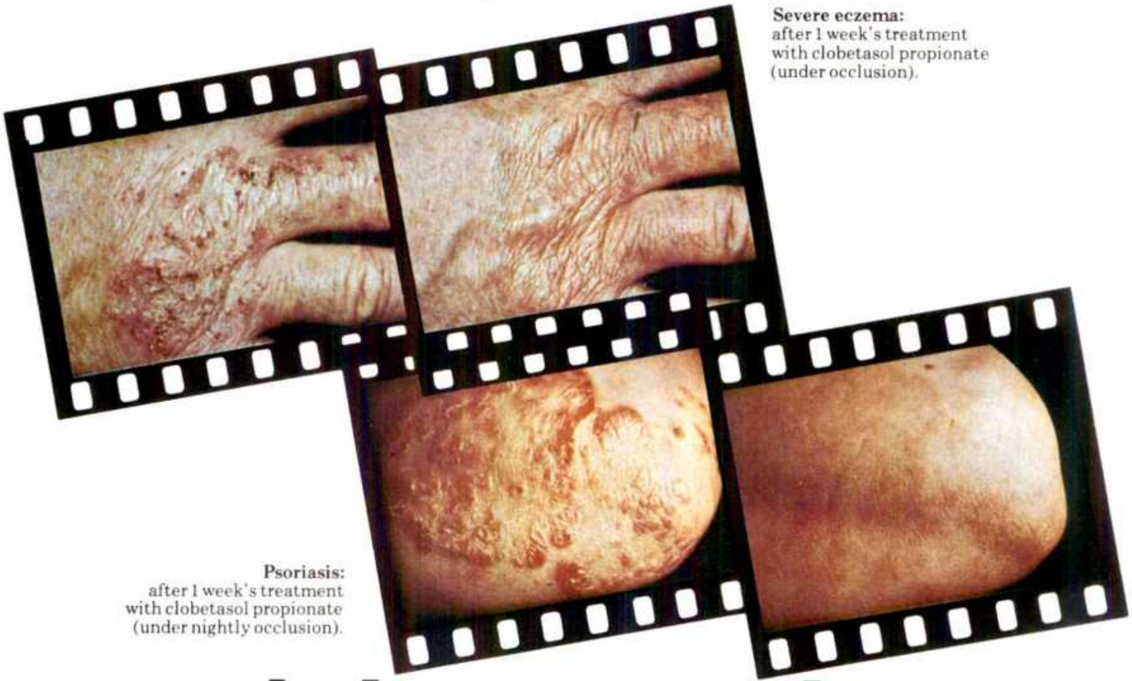

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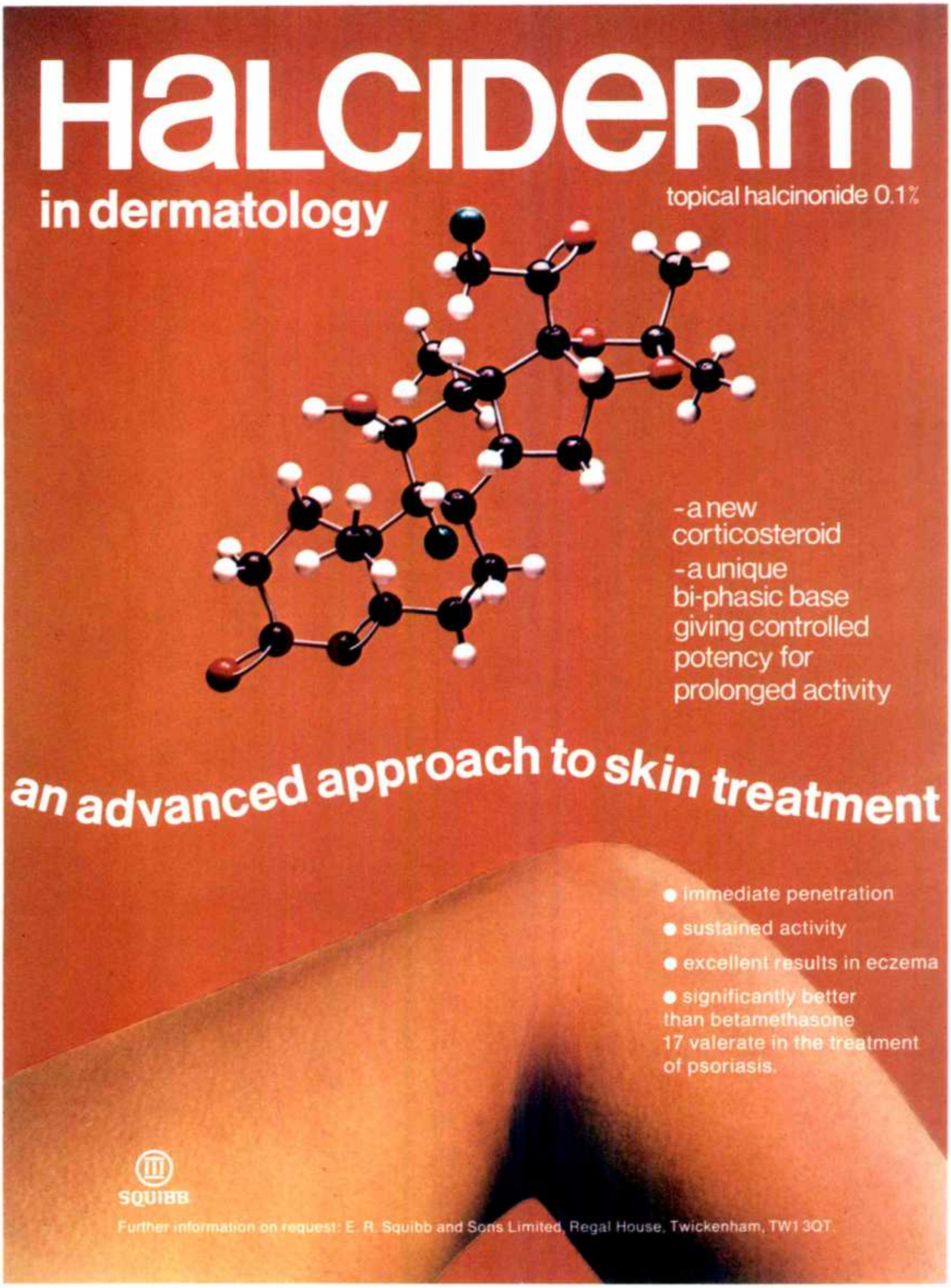

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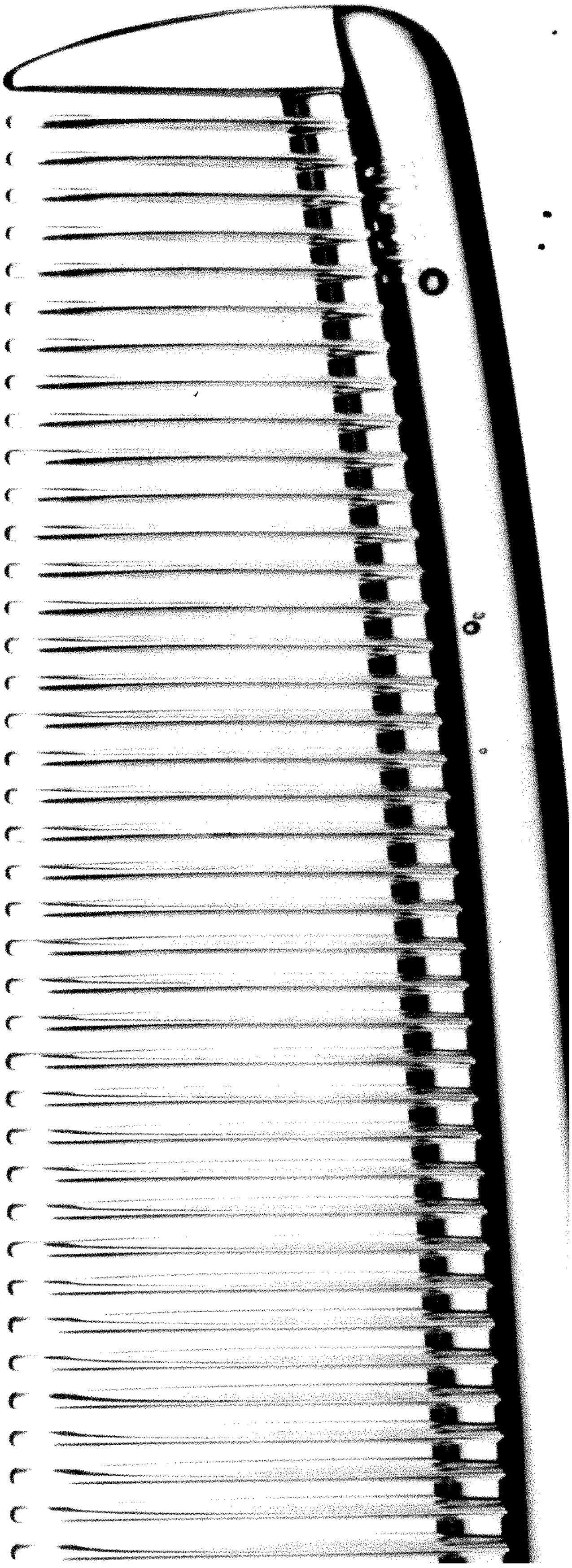

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Langerhans cells in tissue cultures of guinea-pig epidermal cells

C.N.D.CRUICKSHANK AND J.R.COOPER

MRC Unit on the Experimental Pathology of Skin
the Medical School, The University, Birmingham 15

Accepted for publication 31 May 1974

SUMMARY

Dispersed cell cultures of guinea-pig epidermis have been maintained for up to 36 days. The progress of these cultures has been studied by phase contrast microscopy, the adenosine triphosphatase reaction, and cinephotomicrography. Langerhans cells have been identified and their behaviour studied, particularly in relation to keratinocytes.

Langerhans (1868) was the first to describe suprabasal dendritic cells in the epidermis when he impregnated skin preparations with gold salts. These were subsequently named 'high level dendritic cells' by Billingham & Medawar (1953) and have recently been the subject of intense investigation. An extensive review was provided by Wolff (1972), but up to that time there were no reports of successful culture of Langerhans cells. Fritsch & Diem (1972) and Fritsch, Dien & Hönigsmann (1973) demonstrated the survival of Langerhans cells in dispersed cell cultures of guinea-pig epidermis for several days only.

In this study of guinea-pig epidermal cell cultures, we have observed keratinocytes, melanocytes and Langerhans cells for periods of up to 36 days. The techniques of phase contrast microscopy, adenosine triphosphatase reaction (ATPase) and time lapse cinephotomicrography have been used in order to study the behaviour of these different cell types, and their relationship to each other.

MATERIALS AND METHODS

Dispersed cell cultures were prepared from ear skin of albino and variably pigmented guinea-pigs; most of our observations have been on the former. Thin slices of skin, to include as little dermis as possible, were cut, freehand, from the dorsal surfaces of ears using a scalpel. The slices were floated epidermis uppermost on 10 ml of phosphate buffered saline with added calcium and magnesium salts (Dulbecco, A+B) containing 500 international units Amphotericin B and 0·2% trypsin (Nutritional Biochemicals Corporation) and incubated at 37°C for 20 min. The slices were then transferred to a sterile petri dish, and laid flat with the dermis uppermost. The dermis was separated from the epidermis using fine forceps and discarded. The epidermal fragments were then placed in trypsin solution without Amphotericin B and incubated for a further 10 min at 37°C with occasional brisk agitation. The resulting cell suspension was separated from macroscopic tissue fragments by pipetting, and centrifuged at 600 *g* for 3 min. The supernatant fluid was discarded and the cells suspended in growth medium to give a concentration of about 1000 cells/mm^3.

The growth medium consisted of 70% Eagle MEM buffered with Hepes (Biocult Laboratories), and 30% autologous serum; streptomycin and penicillin were added to give a concentration of 100 units of each per ml and L-glutamine 2 mM final concentration. The suspension of cells in medium was placed in chambers (Cruickshank, Cooper & Conran, 1959*) volume 0·16 ml, and incubated at 35°C in the inverted position for 3 days, by which time the viable cells were attached to the glass coverslip. The medium containing non-viable cells and debris was withdrawn from the chamber, 25% of fresh medium was added to it and the mixture was filtered through a 0·45 μ millipore filter in a Swinnex holder and replaced in the chambers (conditioned medium). Similar changes of medium were carried out at 3-day intervals.

The cultures were examined daily by phase contrast microscopy. Cultures at all stages of growth were photographed by time-lapse cinephotomicrography with phase contrast illumination at a magnification of × 100, on to Kodak 'high contrast negative film' to give a relative speed increase of 320 × actual when projected at 16 frames/s.

Cultures at various stages of growth were subjected to the adenosine triphosphatase (ATPase) reaction using the method of Wachstein & Meisel (1963) slightly modified for use with the culture chambers. Culture medium was withdrawn from the chamber, replaced by 10% neutral buffered formalin at 4°C and the chamber placed in a refrigerator at 4°C for 20 min. At the end of this time the wax joint between the coverslip and chamber was brittle and easily broken by forcing a thin razor blade into it. The coverslip was washed with distilled water and inverted, cells down, on to a watch glass of pre-warmed substrate at 37°C for 90 min. The process was completed as in the routine Wachstein & Meisel technique (1963).

The difficulty in correlating phase contrast morphology with the ATPase reaction of individual cells was overcome by the careful use of microscope stage vernier readings and photography (Figs. 1a, b). Once this was accomplished and Langerhans cells identified it was possible to follow the behaviour of these cells in culture. The morphological change induced by the histochemical procedure was striking.

RESULTS

Phase contrast examination of cultures during the first 3 h showed the cells to be round, a few were phase dark but the majority phase light. The ATPase procedure demonstrated numerous positive cells, many with short projections (Fig. 2). By 24 h the original uniform distribution of cells was often replaced by cell aggregations of various sizes. Many round cells remained between the aggregates and some could now be seen flattened on the glass. Time lapse film showed that the peripheral cells of the aggregates had active ruffling movements of a protruded membrane. Some phase dark cells exhibited active movement both into and out of the aggregates. The ATPase reaction now showed the great majority of positive cells to be within the aggregates (Fig. 3).

After the first change of medium at 3 days most of the aggregates, which had only weak attachments, were removed leaving isolated cells spread out on the glass, amongst which keratinocytes and melanocytes could be identified, but only an occasional ATPase positive cell could be found.

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* Now marketed by Sterilin Ltd.

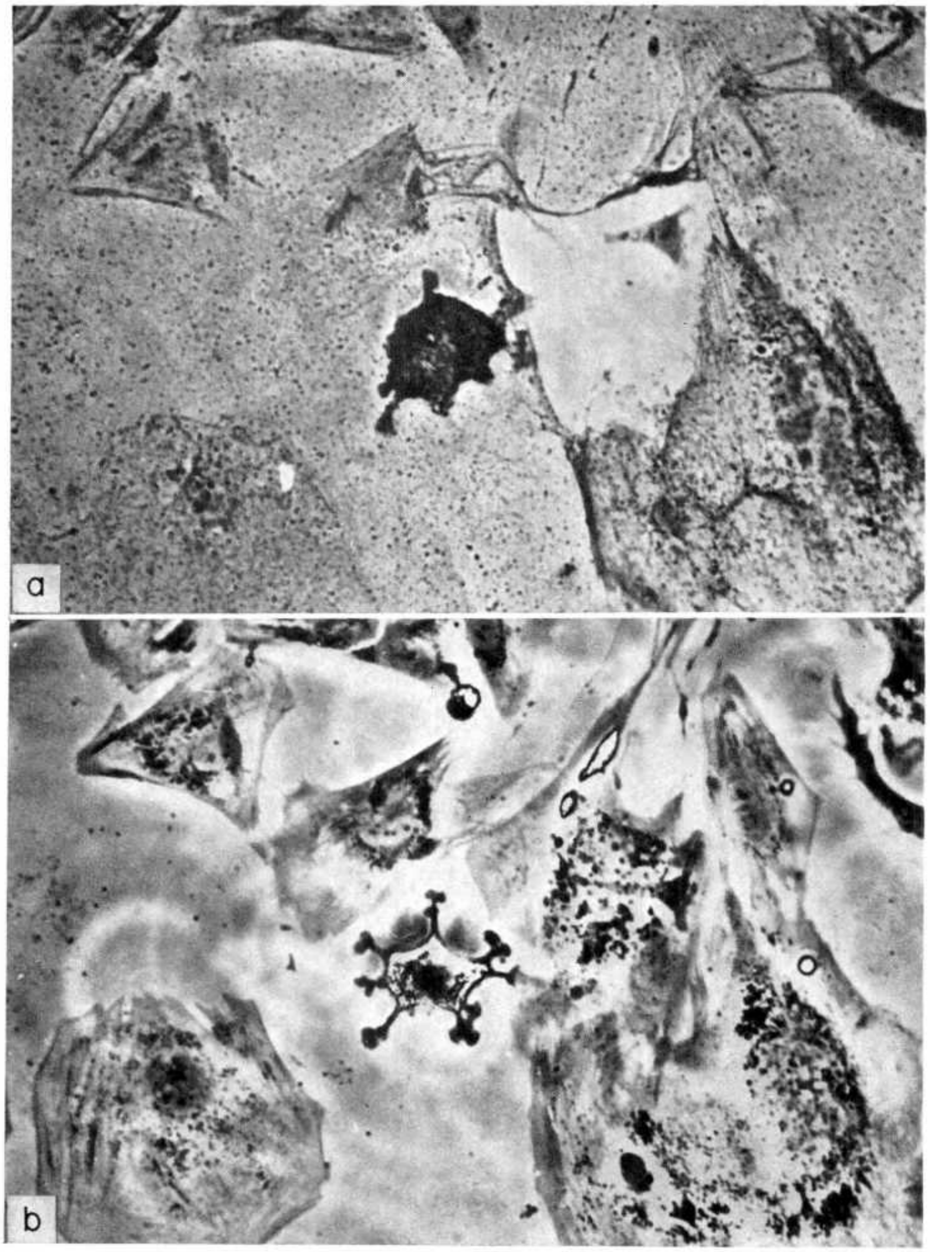

FIGURE 1a. ATPase positive Langerhans cell at 12 days (× 200); (b) the same cell and field by phase contrast (× 200).

The cells, many of which were ATPase positive, contained in the medium removed from the chambers at the first change, were recovered by centrifugation, re-suspended in fresh medium and incubated in fresh chambers. No successful cultures were established from these cells even after incubation of up to 7 days, suggesting that mainly non-viable cells were discarded at the first change of medium.

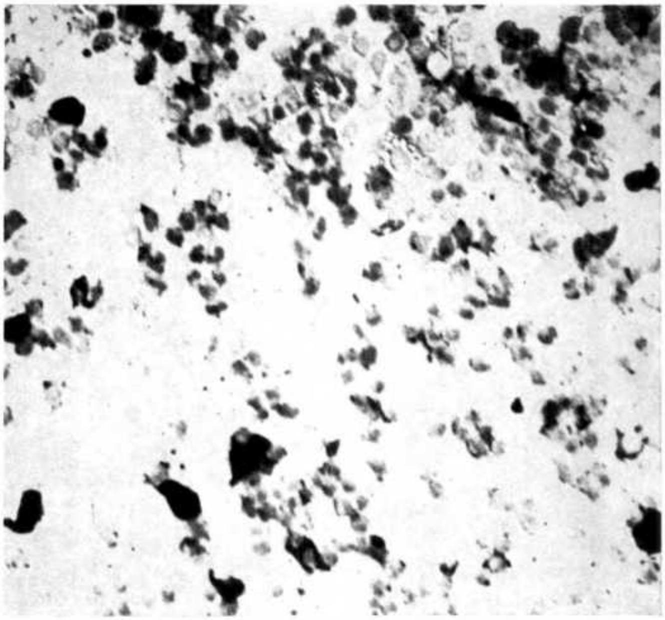

FIGURE 2. Cells in culture at 3 h Eleven Langerhans cells in field. ATPase (× 80).

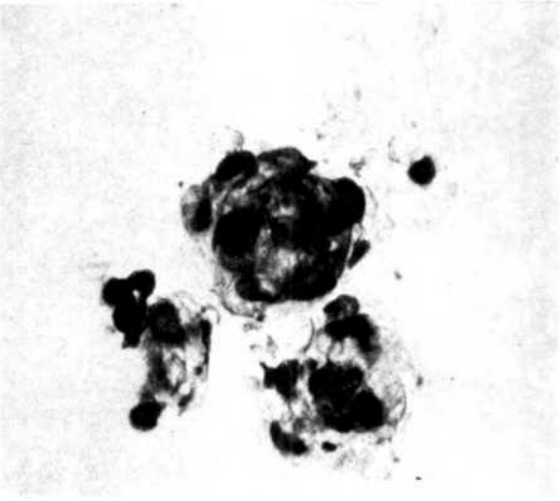

FIGURE 3. Cell aggregate at 2 days containing ATPase positive Langerhans cells (× 200).

Between the third and tenth day, in culture, only a few faintly ATPase positive cells could be seen. By about 10 days there had been considerable growth of keratinocytes to form small colonies, and melanocytes were easily identified. Some clearly ATPase positive cells were present (Fig. 4), some around the periphery of the colonies but predominantly in small lacunae in the sheets of keratinocytes. From 10 days onward, their numbers increased and up to five ATPase positive cells could be present

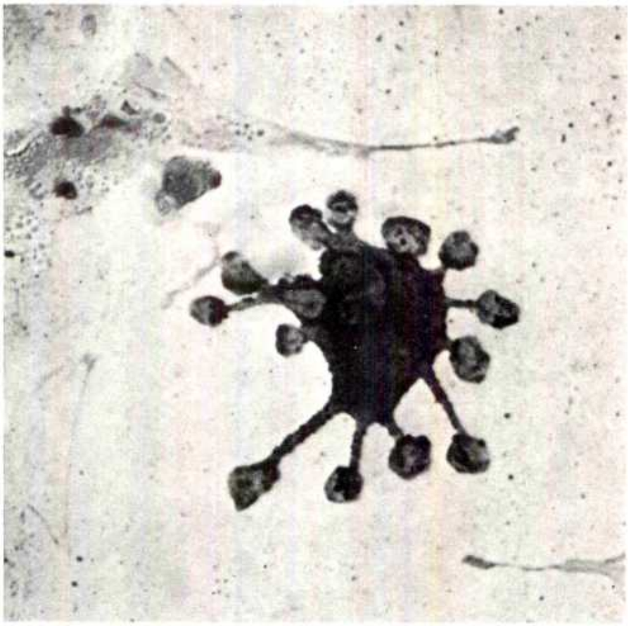

FIGURE 4. Langerhans cell at 10 days. ATPase (× 800).

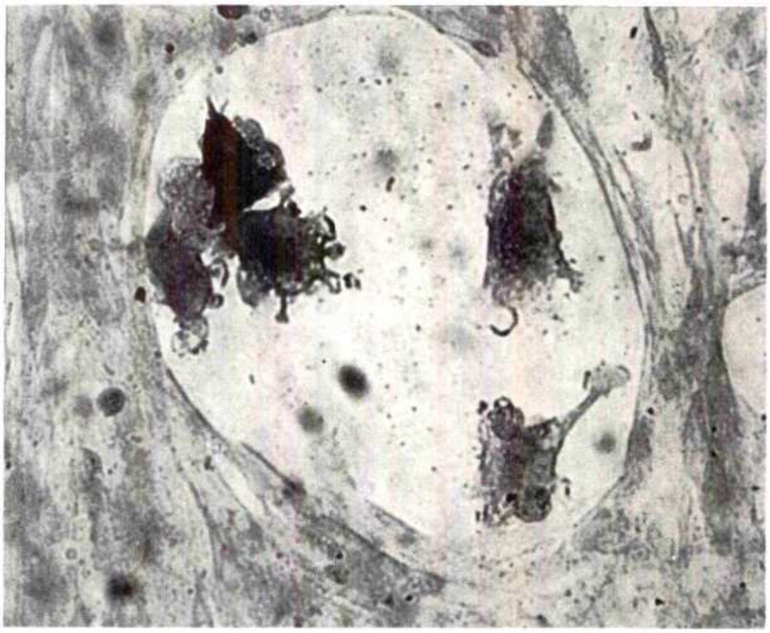

FIGURE 5. Langerhans cells in keratinocyte lacuna at 10 days. ATPase (× 200).

in any one lacuna (Fig. 5). Time-lapse film showed these cells to be very pleomorphic, appearing as rounded cells with cytoplasmic protrusions and a ruffled membrane, dendritic cells with up to twelve processes at the ends of which were phase dense podia, and cells resembling Langerhans cells as seen in tissue sections. Individual cells could have any of the above characteristics at different times. There was very active pseudopodial and dendritic movement but their spatial situation did not change very much. The dendrites could be seen repeatedly touching keratinocytes and frequently extending across several cells of the monolayer, subsequently being withdrawn (Fig. 6). They showed strong contact inhibition and were never seen to move over the surface of the monolayer. There appeared to be an inverse relationship between the numbers of melanocytes and Langerhans cells in different primary cultures. In some films Langerhans cells were seen undergoing division, which also illustrates their pleomorphism (Fig. 7), and in others a slow rounding up and death was observed (Fig. 8). Phase dark amorphous material was present in the cytoplasm and dendrites and was particularly marked during the rounding up process. The possibility should be considered that this material could be imparted to the cells of the granular layer.

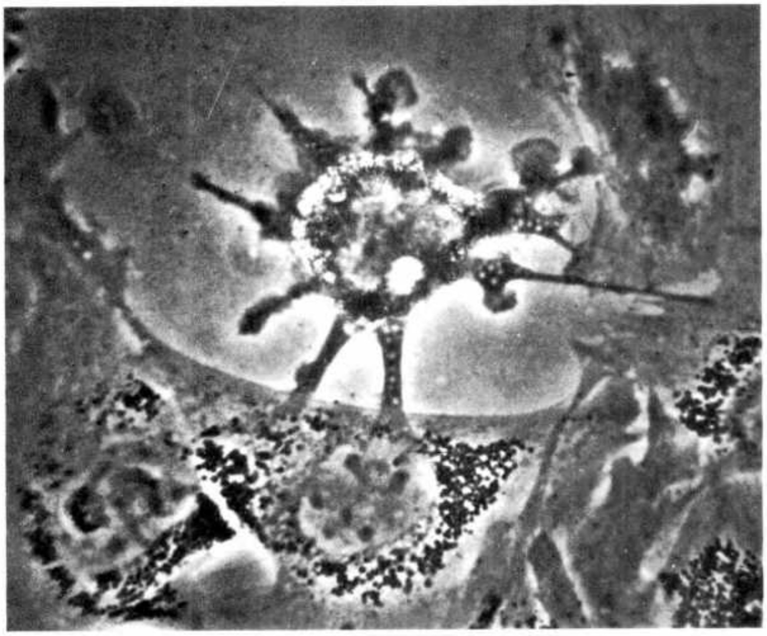

FIGURE 6. Langerhans cell showing intimate contact with keratinocyte in a lacuna at 12 days. Phase contrast (× 800).

By about 15 days the diameter of the keratinocyte colonies had increased and appeared to have reached an optimum size which did not further increase. The keratinocytes frequently contained amorphous hyaline material in their cytoplasm, which may represent early keratin. The number of Langerhans cells increased to a maximum by about the end of week 2 in culture.

Some subcultures were made at 15–20 days and by 10 days later their appearance was similar to the primary cultures at 15–20 days, except that the colonies of keratinocytes were larger and the numbers of ATPase positive cells fewer. When second subculture was carried out, a more confluent monolayer

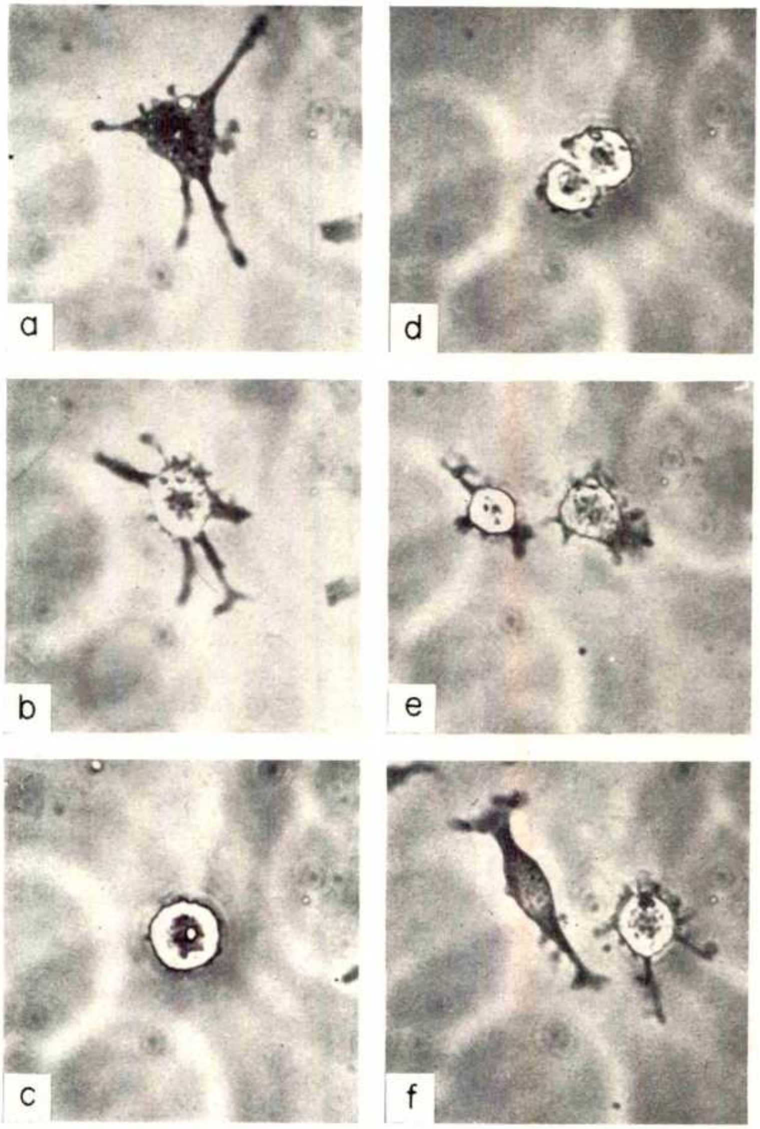

FIGURE 7. Langerhans cell in 12 days culture, time lapse sequence showing mitotic division. a–f, 6 h; c–e, mitotic division estimated duration 3 h, phase contrast (× 180).

of keratinocytes was produced in which only very occasional ATPase positive cells could be found.

Guinea-pig peritoneal macrophages are ATPase positive (personal observation); therefore cultures of these cells were observed and compared with ATPase positive cells in guinea-pig epidermal cultures. Their cultural appearances and behaviour were quite dissimilar.

Preliminary observations of human epidermal dispersed cell cultures have shown ATPase positive cells which have similarities to those in the guinea-pig.

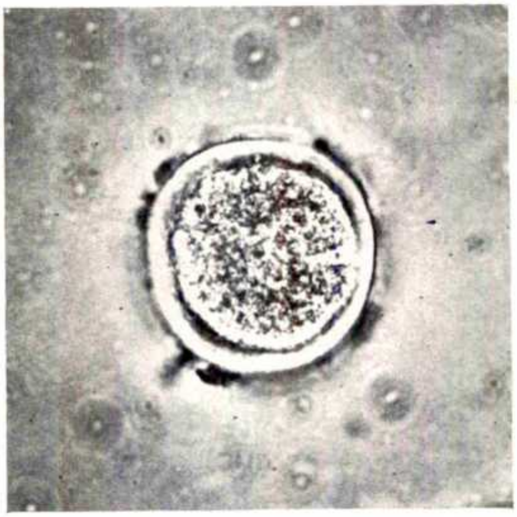

FIGURE 8. Langerhans cell after rounding up, showing granularity and remains of ruffling membrane. Phase contrast (×800).

DISCUSSION

In the extensive review of the literature about the Langerhans cell by Wolff (1972), it is noted that 125 of the 137 references quoted had appeared in the preceding 10 years. It is clear that our notions of the function of the cell are numerous, vague and largely by inference. So far our only criteria of identification have been the facts that the cell is morphologically dendritic *in vivo*, gives a positive ATPase reaction (in the guinea-pig) and contains characteristic ultrastructural racquet-shaped granules. The problem of identification is also complicated by the description of a third type of dendritic cell which ultrastructurally resembles the Langerhans cell in all respects except for the absence of the characteristic granules. The presence of such a cell originally described by Breathnach, Birbeck & Everall (1963) has been amply verified. In this study we have added further criteria of identification by the use of continuous observation of epidermal cell cultures by time-lapse cinephotomicrography, combined with a comparison of phase contrast appearances with the ATPase reaction.

In those cultures in which there is early aggregation of cells, dendritic ATPase positive cells can be seen in the centres of the aggregates and there is ultimately a poor yield of Langerhans cells. This suggests that the Langerhans cell may be acting as an organizer, and we have shown that many are lost as the clumps are washed out when the medium is changed. The best yield of ATPase positive cells occurs when there is minimal early aggregation. The second point is that between 3 and 10 days only faintly positive cells are found; but, subsequently, strongly positive, pleomorphic cells are visible and it is at this stage that divisions occur. It seems likely that the degree of the ATPase reaction varies with time in culture, and possibly is affected by intercellular reactions with keratinocytes. Wolff (1972) discusses the problem of interpreting positive or negative staining of Langerhans cells by the ATPase reaction. The later positive cells may arise from precursor cells either from the epidermis or from dermal contamination In any event, our studies would suggest that there is a population of cells, probably epidermal, which do not necessarily exhibit the ATPase positive reaction or typical *in vivo* morphology at all stages. These could be the 'third cell' of electron microscopy.

In later cultures the cell–cell interactions of the Langerhans cells in the lacunae of firmly established monolayers is of interest. They have intimate contact with groups of keratinocytes which lends support to the hypothesis of a 'Langerhans cell–keratinocyte unit'. Here they seem also to act as organizers as judged by the immediacy of the cytoplasmic reaction of the keratinocytes to contact with their podia.

The large number of ATPase positive cells present during the first three days of culture is greatly reduced by the first change of culture medium. Cytologically these cells are similar to the ones illustrated by Fritsch *et al.* (1973) but are removed from the chambers at the first change of medium because they do not adhere to glass. Our unsuccessful attempts to culture these cells suggests that both Langerhans cells and keratinocytes are not viable after aggregation. An electron microscopy study of these cell aggregates has shown the majority of keratinocytes to be cornified (Constable *et al.*, 1974).

It has been suggested from observations of film which we have previously shown, that the Langerhans cells which we have described might be macrophages. Although guinea-pig peritoneal macrophages are ATPase positive, there are distinct differences between the two cell types. The Langerhans cell is much larger, has a granular cytoplasm, and contains few phagocytic vacuoles. Its form of movement is quite different, being much more static and indulging in considerable pseudopodial movement and showing contact inhibition, whereas macrophages do not display contact inhibition and move freely through and over monolayers of keratinocytes. We have never seen guinea-pig macrophages divide.

The inverse relationship between the numbers of melanocytes and Langerhans cells in different primary cultures is interesting in that it has previously been observed in various dermatoses both histologically and by electron microscopy (Job, Nayar & Narayaman, 1972; Mann & Cowan, 1973; Wolff, 1972), but its significance is unknown.

Cohen & Szabo (1968) described non-pigmented dendritic cells in their cultures but regarded them as melanoblasts. We have previously observed such cells and described them as non-pigmented dendritic cells (Cruickshank *et al.*, 1960). In hindsight, it is unlikely that these were Langerhans cells.

There are several possible reasons for our success in cultivating Langerhans cells. These include (1) the use of Hepes buffered medium which may well maintain an optimum pH during the critical period when the cells are becoming established in culture; (2) 'conditioned medium' may contain a factor which encourages the vigour of cultures; (3) the excellent optical characteristics of the culture chambers; (4) the additional parameter of time-lapse cinephotomicrography.

ACKNOWLEDGMENTS

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Patch testing in the rabbit using a modified human patch test method

APPLICATION OF HISTOLOGICAL AND VISUAL ASSESSMENT

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SUMMARY

A 5 h, semi-occlusive human patch test repeated daily for 5 days, was modified for use in the rabbit Using this method, the relative irritancy of three cosmetic samples of known human irritancy was determined in the rabbit.

In addition, attention was paid to assessment methods and a detailed histological scoring system is described as well as two methods of visual assessment. In order to compare the effectiveness of these methods, the reaction to a series of concentrations of sodium lauryl sulphate was examined.

All three assessment methods gave similar results and the merits of combining histological and visual assessment are discussed.

Comparison with human results showed agreement in the case of the most irritant cosmetic sample, but revealed a reversal of the relative irritancy of the other two cosmetic samples. The implications of this in the testing of mild irritants are discussed.

Although the rabbit patch test devised by Draize, Woodard & Calvery (1944) gives a reasonable prediction of the human reaction to strong irritants, its predictive value has been questioned in the case of mild irritants (Carter & Griffith, 1965; Phillips *et al.*, 1972; Lansdown, 1972).

Differences in technique could be a contributory factor to the poor predictive value of rabbit tests for mild irritants. Thus the Draize test in the rabbit involves a single 24 h treatment, whereas in human tests treatment is often continued for 2–3 weeks (Lanman, Elvers & Howard, 1968; Phillips *et al.*, 1972). Phillips *et al.* demonstrated that if the protocol of the Draize test was used in human testing, mild irritants could not be detected.

In this paper the results of applying a 5-day human patch test protocol to rabbit testing are presented, using a modification of the human test of Uttley & van Abbé (1973), which in turn is based on the method of Finkelstein, Laden & Miechowski (1963).

Assessment in the Uttley and van Abbé method is based on a visual estimation of erythema, with oedema and blistering as subsidiary criteria. Since visual assessment tends to vary from laboratory to laboratory (Weil & Scala, 1971), we have devised a histological scoring system and compared this with visual assessment using a series of concentrations of sodium lauryl sulphate.

By testing cosmetic samples previously tested in man by the Uttley and van Abbé method, rabbit and human reactions could be compared.

MATERIALS AND METHODS

Animals. Female New Zealand white rabbits (2–3 kg wt) were clipped dorsally on a Friday for use the following Monday, only rabbits free of hair growth being used (see Rony, Cohen & Schaffner, 1953; and Chase, 1954 for information on hair growth cycles). During patch testing the rabbits were held in restrainer boxes.

Test substances. A range of concentrations of sodium lauryl sulphate of 0·0096–2·4% wt/vol were made up in 0·1 M phosphate buffer pH 8. In addition to sodium lauryl sulphate, three cosmetic samples (A, B and C) were tested. Each concentration of sodium lauryl sulphate and each cosmetic sample was tested in duplicate, duplicates being tested on separate rabbits.

The controls used were pH 8 buffer alone, arachis oil and dry patches.

Patch testing method

Absorbent patches were made from the backing material of 'Melolin'* perforated film absorbent dressings; this material was more spongy than lint and gave better patch contact. The patches, 1·5 cm^2, were backed with occlusive 'Blenderm'† tape and supported 2 cm apart on 11 cm lengths of 'Dermicel'‡ surgical tape 7·5 cm wide. There were six patches to each piece of 'Dermicel' tape, the whole being referred to as a multiple patch. As 'Dermicel' does not stick well to rabbit skin, strips of 'Sleek'* plastic adhesive 1 cm wide were placed between the patches.

To each absorbent patch, 0·2 ml of test or control substance was applied. The multiple patch was placed mid-dorsally in a longitudonal direction so that each patch was equidistant from the backbone. When positioned on the rabbit, a border of 2 cm wide 'Sleek' was placed round the multiple patch to stick down the edges, wrinkling of the skin being prevented by a wire rectangle attached to the 'Sleek' border.

The patches were in place for 5 h every day for 5 days. In order that accurate relocation of the patches was achieved the corners of each patch area were marked with a fibre tip pen.

Assessment methods

Visual (A and B). Visual assessment was made 30 min after removal of the patches on each of the 5 days. Assessment was made on a scale based on that of Uttley & van Abbé (1973) but modified to take into account the different pattern of response occurring in the rabbit.

Scale 0, no reaction; 1, just visible erythema; 2, definite slight erythema; 3, moderate erythema, no oedema; 4, moderate erythema, some oedema or strong erythema, no oedema; 5, strong erythema, moderate oedema; 6, strong erythema and oedema, slight cracking and exudation; 7, slight ulceration (opening cracks); 8, ulceration.

(A) *Non-biased cumulative visual score.* This score was obtained by adding together the daily scores for the 5 days, giving a maximum score of 40.

(B) *Biased cumulative visual score.* Some human patch test methods (e.g. Lanman *et al.*, 1968) use the time of reaction to develop, rather than the degree of response, as a measure of irritation.

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* Supplied by Smith & Nephew Ltd, Hull, England. † Supplied by the 3M Company, London.

‡ Supplied by Johnson & Johnson, Ltd, Slough, England.

In order to take both the degree and speed of response into consideration a biasing system has been devised to emphasize the earlier scores. This system is outlined below.

$$\begin{array}{c} \text{Day 1 Score} \times 5 \\ + \\ \text{Day 2 Score} \times 4 \\ + \\ \text{Day 3 Score} \times 3 \\ + \\ \text{Day 4 Score} \times 2 \\ + \\ \text{Day 5 Score} \times 1 \end{array} \quad \frac{\text{Total}}{3} = \text{Biased cumulative visual score}$$

As with the non-biased visual score the maximum score is 40.

(C) *Histological assessment*. The rabbits were killed by cervical dislocation 1 h after the last 5 h treatment period and the patch areas were fixed in 10% buffered formalin. After paraffin embedding, sections were cut at 4–6 μm parallel to the direction of hair growth and stained with haematoxylin and eosin.

Histological assessment was based on both epidermal and dermal changes, with emphasis on easily quantifiable features. The scoring system is given below.

	Score	Maximum score
(a) *Epidermal thickening*		
1½–3 × normal thickness in places	1	5
1½–3 × normal thickness generally	2	
3–4 × normal thickness in places	3	
3–4 × normal thickness generally	4	
More than 4 × normal thickness	5	
(b and c) *Hyperkeratosis*		
(b) Increase in cell layers of stratum granulosum		
1 cell layer thick	0	3
2 cell layer thick	1	
3 cell layer thick	2	
4 or more cell layers thick	3	
(c) Thickening of stratum corneum		
Thickening only	1	2
Thickening plus surface debris	2	
(d, e, f and g) *Other epidermal changes*		
(d) Absence of stratum granulosum		
Focal	1	2
General	2	
(e) Parakeratosis		
Focal	1	2
General	2	

	Score	Maximum score
(f) Isolated cell damage		
Stratum spinosum only	1	2
Basal layer also	2	
(g) Spongiosis		
Slight	1	4
Extensive	2	
Microvesicle formation	3	
Blister formation	4	
(h) *Destruction of epidermis*		
Focal	15	30
$\frac{1}{4}$ of sectioned area	18	
$\frac{1}{2}$ of sectioned area	20	
$\frac{3}{4}$ of sectioned area	25	
Whole of sectioned area	30	
(i and j) *Dermal changes*		
(i) Hyalinization of collagen		
Focal	1	4
$\frac{1}{2}$ sectioned area	2	
$\frac{3}{4}$ sectioned area	3	
Whole of sectioned area	4	
(j) Infiltration of dermis		
Slight–focal	1	6
Slight–diffuse	2	
Moderate–focal	3	
Moderate–diffuse	4	
Severe–focal	5	
Severe–diffuse	6	

Totalling system

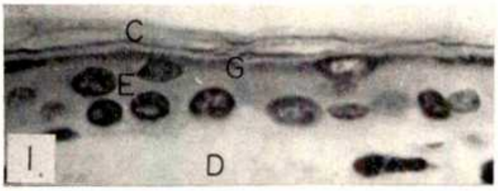
Histological score $=(a+b+c+d+e+f+g)+(i+j)$, or $h+(i+j)$ whichever is the larger.

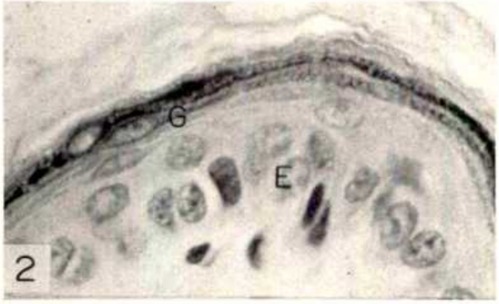
Further details on histological scoring

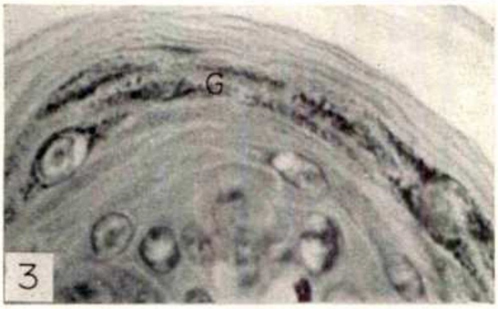
(a) *Epidermal thickening* (*Acanthosis*) The thickness of the epidermis was measured from the base of the basal layer to the outside of the granular layer (i.e. excluding the stratum corneum) with an eyepiece graticule. Cell layers were not counted here as the cells of the stratum spinosum do not form clear layers. Measurements were only made in vertically sectioned regions of the epidermis, tangentially sectioned regions being recognized by the wide appearance and diffuse borders of the granulosum cells.

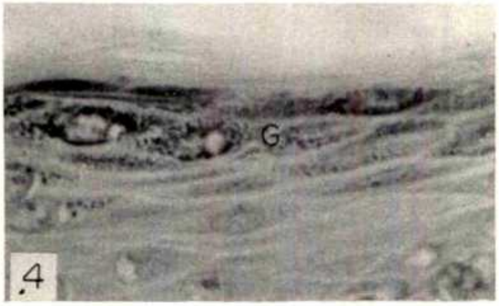
The score for epidermal thickening was obtained by comparison of the epidermal thickness of the test area with that of the surrounding area.

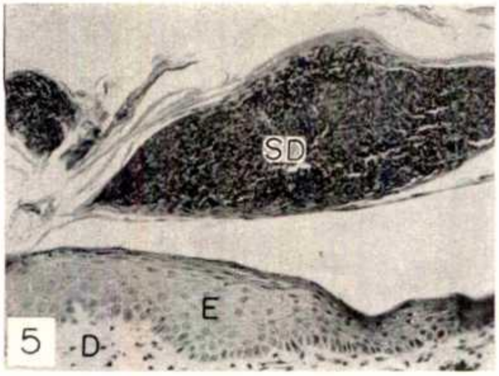
(b) *Stratum Granulosum.* The stratum granulosum of normal rabbit skin consists of a single layer of flattened cells joined end to end (see Fig. 1). When extra granulosum cell layers were present, the cells of one layer usually alternated with those of adjacent layers (Figs. 2–4). The score in this category refers to the region where the greatest number of layers was found.

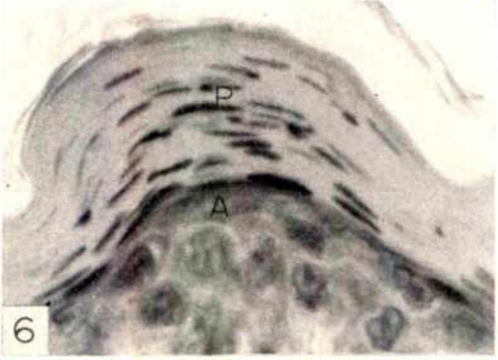












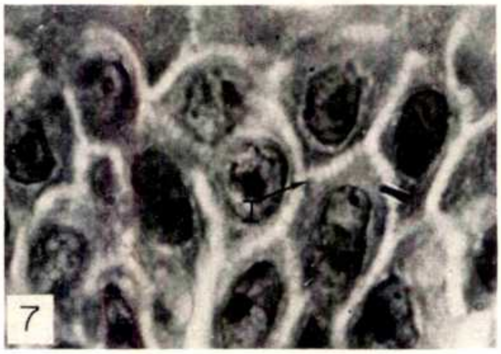


FIGURE 1. Normal rabbit skin showing (E) basal and spinosum layers of epidermis, (G) stratum granulosum one cell thick, (C) stratum corneum and (D) dermis (H&E × 800).

FIGURE 2. Sodium lauryl sulphate treated rabbit skin showing (G) stratum granulosum 2 cells thick and (E) epidermis thickened (H&E × 800).

FIGURE 3. Sodium lauryl sulphate treated skin showing (G) stratum granulosum 3 cells thick (H&E × 800).

FIGURE 4. Sodium lauryl sulphate treated skin showing (G) stratum granulosum 4 or more cells thick (H&E × 800).

FIGURE 5. Sodium lauryl sulphate treated skin showing (SD) surface debris, (E) epidermis and (D) dermis (H&E × 130).

FIGURE 6. Skin treated with Sample (B) showing (P) parakeratosis and (A) absence of stratum granulosum (H&E × 800).

FIGURE 7. Spongiosis showing (I) increase in intercellular space (H&E × 1300).

(c) *Cornified Layer.* Thickening of the cornified layer is difficult to quantify as it is sometimes flaky and difficult to define at the edge; it is also prone to distortion during sectioning. For these reasons not much emphasis was placed on this feature. Nuclear debris was seen on the surface of the stratum corneum following 5 days of sodiumlauryl sulphate treatment (Fig. 5). Sequential histological examination of the reaction to sodium lauryl sulphate (Ingram, unpublished experiments) suggests that this surface debris constitutes the remains of an early dermal reaction which has been undercut by the surrounding epidermis. This indicates that a new stratum corneum has been formed and a score of 2 was given therefore for the presence of surface debris.

(d) *Absence of stratum granulosum and* (e) *Parakeratosis.* 'Absence of the stratum granulosum' refers to an apparent disappearance of the keratohyalin granules as visualized by H & E staining, whereas parakeratosis is a retention of nuclei in the cells of the stratum corneum. Both these features suggest alterations in keratin metabolism and are usually found together (see Fig. 6).

(f) *Isolated cell damage.* Epidermal cells with pyknotic nuclei were sometimes seen in the stratum spinosum and basal layer (see Fig. 8).

(g) *Spongiosis (intercellular oedema).* Spongiosis could be recognized by spaces and stretched-out cytoplasmic bridges between epidermal cells (see Fig. 7). More severe forms of the same reaction are microvesicle and blister formation.

(h) *Destruction of epidermis.* This is represented by the death or breakdown of the granulosum, spinosum and basal layers of the epidermis (see Fig. 9). This reaction is considered more severe than the epidermal changes a–g. Scores for the various degrees of epidermal destruction were therefore made to exceed the combined scores of the other epidermal changes. Although the lowest epidermal destruction score is less than the maximum possible score for epidermal changes, in practice this maximum is never achieved.

(i) *Hyalinization of collagen and* (j) *Infiltration of dermis.* Two features can occur in the dermis following topical application of substances to the skin; these are hyalinization of collagen (Figs. 10–11) and infiltration of inflammatory cells (Figs 12–14). Infiltration of inflammatory cells may follow hyalinization of collagen or may occur on its own.

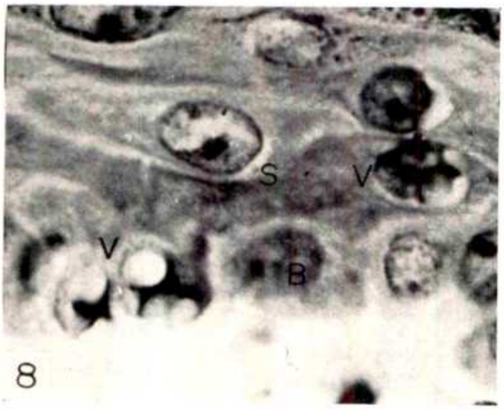
Totalling System

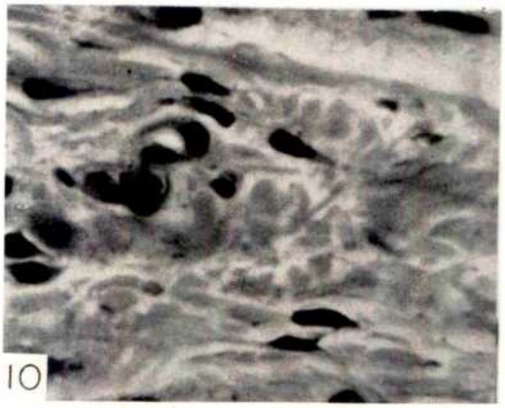
As shown in the scoring system details, the histological score was obtained by adding the sum of the scores for the epidermal features (a–g) to the dermal score (i + j). Where destruction of the epidermis had taken place the score for this alone (h) was combined with the dermal score. As with the two visual scoring systems the maximum score was 40.

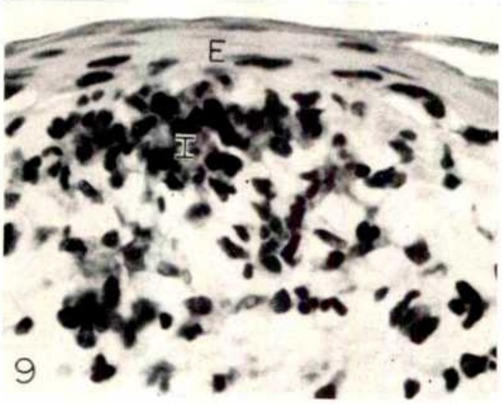
RESULTS

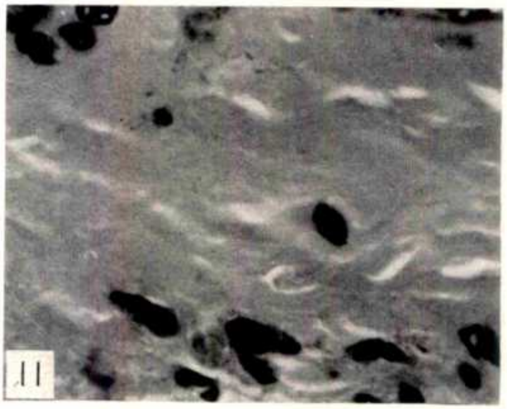
Controls. No visual reaction was observed with any of the control patches. Histological scoring gave no values greater than 2 for buffer or dry controls, the mean score of the buffer controls being 1. Arachis oil gave a histological score of 5, showing slight acanthosis and hyperkeratosis.

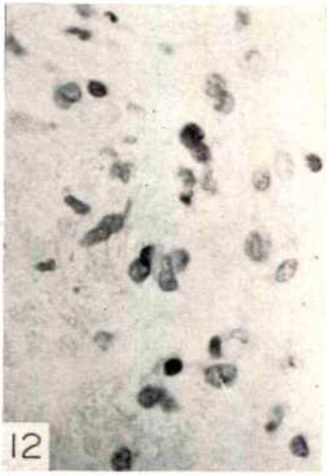
Reactions to sodium lauryl sulphate. No reaction was observed visually with 0·0095% sodium lauryl sulphate. In response to 0·038–2·4% sodium lauryl sulphate the first signs of erythema were usually

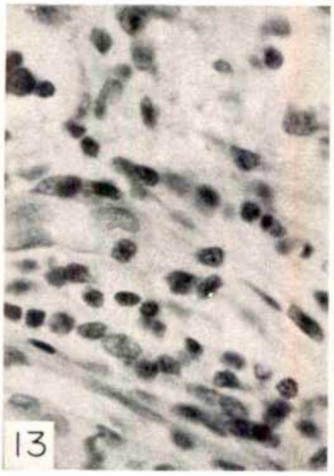












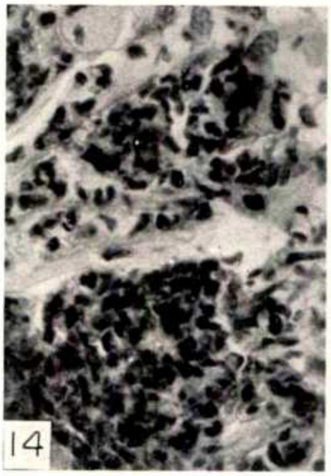


FIGURE 8. Sodium lauryl sulphate treated skin showing (V) vacuolated nuclei in the basal layer (B) and the spinosum layer (S) of the epidermis (H&E × 1300).

FIGURE 9. Sodium lauryl sulphate treated skin showing (E) dead epidermis and (I) cellular infiltration (H&E × 800).

FIGURE 10. Normal dermis showing well-defined collagen fibres (H&E × 800).

FIGURE 11. Sodium lauryl sulphate treated skin showing hyalinization of collagen (H&E × 800).

FIGURE 12. Sodium lauryl sulphate treated skin showing slight dermal infiltration (H&E × 600).

FIGURE 13. Moderate dermal infiltration (H&E × 600).

FIGURE 14. Severe dermal infiltration (H&E × 600).

apparent just after the first 5 h treatment. The maximum degree of erythema and oedema resulting from sodium lauryl sulphate treatment was usually reached on day 2 or day 3, after which time oedema decreased and erythema became darker and less pronounced.

The two types of cumulative 5 day visual scores (non-biased and biased) both revealed a general increase in response in proportion with the log. of the concentration (Figs. 15a, b).

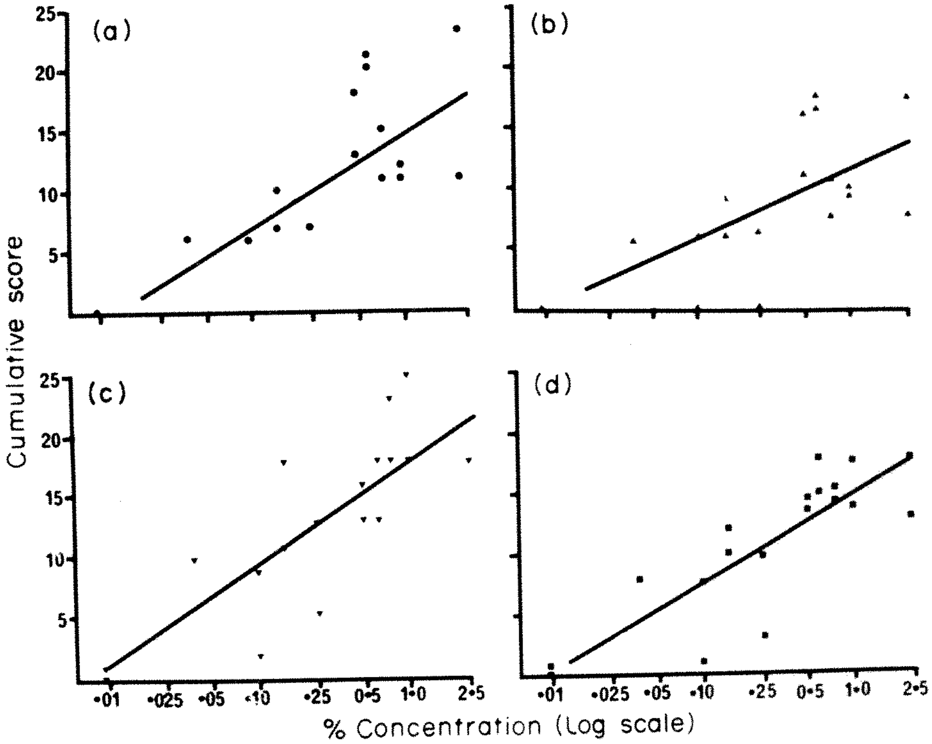


FIGURE 15. Dose-response relationship for sodium lauryl sulphate
Graph a, Non-biased visual scoring; Graph b, Biased visual scoring; Graph c, Histological scoring; Graph d, Mean of histological and biased-visual scoring.

Histological examination after the 5-day treatment schedule revealed no reaction to 0·0095% sodium lauryl sulphate. In most patches treated with 0·038–0·1% sodium lauryl sulphate slight acanthosis and hyperkeratosis were evident, together with a slight infiltrative response; no destruction of the epidermis was found. Between the concentrations of 0·15% and 0·5% marked acanthosis and hyperkeratosis were evident, together with signs of epidermal damage (spongiosis and isolated cell damage). Epidermal destruction of the lowest grade was found in one patch in this range. Slight to moderate dermal infiltrations were evident in most patch areas in this range. Sodium lauryl sulphate above 0·6% produced various degrees of epidermal destruction with moderate to strong dermal infiltration. Only one patch in the 0·6–2·4% range failed to show epidermal destruction and this showed similar features to the 0·15–0·5% range.

Parakeratosis and loss of the granular layer were not usually found after 5-day exposure to sodium lauryl sulphate and hyalinization of dermal collagen was seen only in short term (1–3 day) reactions to concentrations above 0·5% (unpublished results).

As with visual observations there was a general increase in histological score proportional to the log, of the concentration of sodium lauryl sulphate.

Comparison of Scoring Methods. The reactions to the various concentrations of sodium lauryl sulphate, assessed by non-biased visual, biased visual and histological scoring are shown graphically in Fig. 15 a–c. In order to compare the three scoring methods, regression lines were plotted. Table 1 shows the regression scores for three dose levels together with their standard error. The biased visual scoring system gave lower scores than the non-biased visual, with a correspondingly reduced standard error. The histological scoring system gave a higher score level and a slightly lower standard error than both visual methods. Although the differences in standard error between the three assessment methods were very small, the association of the highest score level with the lowest standard error in the histological method suggests that this system may be more sensitive than visual assessment. Confirmation of this would require larger scale experiments than described here. Fig. 15d and Table 1 give details of the mean of the histological and biased visual scores for the various dose levels of sodium lauryl sulphate; the standard error was slightly less than the individual scoring methods.

TABLE 1. Regression scores of sodium lauryl sulphate reactions at low, medium and high dose levels

Concentration of Sodium lauryl sulphate	Scoring method	Regression score	Standard error
0·1%	A	6·7	1·4
	B	5·8	1·2
	C	9·5	1·1
	D	7·8	1·0
0·5%	A	12·2	1·2
	B	9·9	1·1
	C	15·4	1·0
	D	13·5	0·9
2·4%	A	17·5	2·0
	B	13·9	1·7
	C	21·2	1·7
	D	19·1	1·4

A, Non-biased visual; B, Biased visual; C, Histological; D, Mean of biased visual and histological.

Reactions to Cosmetic Samples. Visually the reactions to the cosmetic samples appeared later than the reactions to sodium lauryl sulphate. With sample (C), reactions began on day 2, with sample (B) they began on day 3 and with a sample (A) a minimal reaction started on day 3 or 4. In no cases did the visual reaction to the cosmetic samples start to subside during the 5 days of treatment.

Histologically, sample A produced marked acanthosis and hyperkeratosis together with a slight to moderate dermal infiltration. In addition to this, slight parakeratosis and disappearance of the granular layer was evident in a small area of one patch, but not the other. Sample (B) produced marked acanthosis and although some hyperkeratosis was present, parakeratosis and disappearance of the granular layer were evident in several places. A moderate dermal infiltration was present. Sample (C) produced epidermal destruction and moderate dermal infiltration.

Table 2 shows the scores obtained in response to each cosmetic sample by the three scoring systems, together with the mean scores for the combined histological and biased visual methods. With sample (A) visual scores were very low, whereas moderate histological scores were obtained. With sample (B) moderate visual and histological scores resulted and with sample (C) high visual and histological scores were obtained. With all scoring methods the irritancy ranking was (A) less irritant than (B and (B) less irritant than (C).

TABLE 2. Cosmetic reactions assessed by the various scoring methods

Substance	Scoring method			Mean of histological and biased visual scores	Mean of duplicate H. & B. V. scores
	Non-biased visual	Biased visual	Histological		
Sample A	2	1·0	11	6·0	7·6
	4	2·3	16	9·2	
Sample B	13	7·3	16	11·7	11·7
	12	7·3	16	11·7	
Sample C	17	12·0	22	17·0	18·5
	21	16·0	24	20·0	

DISCUSSION

Histology as an aid to assessment

Visual scoring usually relies mainly on dermal changes and may therefore give an incomplete picture; histological assessment on the other hand enables epidermal changes to be taken into account as well. The reaction to cosmetic sample (A) emphasizes this point, epidermal changes being observed histologically while little reaction was observed visually. Histological scoring may also be more objective than visual methods.

The histological assessment methods described by other workers are either based on a single criterion (e.g. Schaaff & Gross, 1953) or do not have enough categories for detailed grading of reactions (e.g. Hunter, Brown & Ferrigan, 1966). The histological assessment method described in our paper give a score range of 1–40, thus allowing comparisons as detailed as with conventional visual scoring methods. By scoring each feature separately and totalling the scores for all features, this method allows comparison of irritants which affect the various histological structures to differing extents.

Comparison of histological with visual scoring systems revealed a similar dose-response relationship in response to sodium lauryl sulphate, the histological method being perhaps slightly more sensitive.

The main limitation of the histological scoring system is that progression of the irritant reaction cannot be followed in the same way as with visual assessment. By taking the mean of the histological and biased visual scores, the early and the late changes can be taken into account and thus this combination was considered to give the best indication of the overall reaction.

Cosmetic irritancy in rabbit and man

The irritancy ranking of the cosmetic samples in the rabbit was the same by all methods of scoring, though the irritancy of samples (A) and (B) was closer with histological scoring.

In human testing sample (A) was more irritant than sample (B), whereas in the rabbit the reverse was the case. This agrees with the findings of Carter & Griffith (1965) who found that when compounds of different natures were ranked for irritancy in the rabbit and man, the order may be different in the two species. The assumption that a substance which has proved just acceptable in man is a good standard for any type of substance in rabbit testing is thus erroneous; it is only a good standard for similar substances.

Samples (A) and (B) were both regarded as acceptable as a result of human testing; thus a substance similar to sample (B) might be regarded as acceptable if it produced a reaction in the rabbit of 11·7 (histological and biased visual). If however a substance similar to sample (A) produced a reaction of 11·7 it might be unacceptable since 11·7 is greater than the reaction to sample (A).

Sample (C) was the most irritant of the cosmetic samples in human testing and was regarded as unacceptable. As regards sample (C), therefore, the rabbit tests were in agreement with human tests, sample (C) giving a considerably higher score level than the other two samples in the rabbit. The reactions to the three cosmetic samples in the rabbit were consistently greater than those in man under similar test conditions.

Concluding remarks

This work indicates that the human patch test of Uttley & van Abbé (1973) can, with minor modifications, be applied successfully to the rabbit. The use of histological assessment in conjunction with visual assessment provided additional information and could result in a less subjective and more sensitive evaluation. To establish such a test routinely in the rabbit it would be desirable to test many types of acceptable cosmetics in order to define the acceptable range for each type of compound. To define of acceptable limits for each type of compound will be the aim of subsequent work.

ACKNOWLEDGMENT

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British Journal of Dermatology (1975) **92,** 143

Photochemistry of tetrachlorosalicylanilide and its relevance to the persistent light reactor

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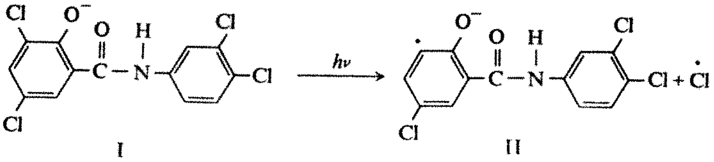
SUMMARY

The photochemistry of 3,5,3′,4′-tetrachlorosalicylanilide has been studied in solution under carefully controlled conditions. When irradiated in a buffered solution of pH 7·4 (physiological pH), three atoms of chlorine are liberated from the molecule instead of one as suggested by earlier photochemical work. From this observation a mechanism is proposed to explain the long-term photobiological effect of this compound in skin i.e. that of the persistent light reactor.

The photobiological effect on the skin of 3,5,3′,4′-tetrachlorosalicylanilide (T4CSA), originally employed as an antibacterial agent in soap, has been recognized for over a decade (Wilkinson, 1961). A curious feature, and one that has as yet no satisfactory explanation, is that the skin can remain highly sensitive to sunlight for months or even years after termination of all known contact with the sensitizer (Willis & Kligman, 1968). A patient with this long-term light sensitive condition has been described as a 'persistent light reactor' (Jillson & Baughman, 1963).

T4CSA can have both short-term and long-term photobiological effects on the skin and the short-term effect appears readily explicable in terms of its reported photochemistry (Jenkins, Welti & Baines, 1964; Coxon, Jenkins & Welti, 1965). In aqueous alcoholic solutions of neutral pH, T4CSA is present in both non-ionic and anionic forms (pK = 5·6) but it is only the anionic form I (see below) that is photochemically active to near ultra-violet radiation.



(1)

The free radical II formed on photolysis has been shown to combine readily with γ-globulin and protein in rat serum (Jenkins *et al.*, 1964). It would thus appear likely that II is readily capable of combining with the protein of human skin. However the long-term sensitizing effect, as seen in the persistent light reactor, cannot be satisfactorily explained by this mechanism of a single photolytic

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step except, of course, by assuming that T4CSA is retained in the skin for very long periods of time (Willis & Kligman, 1968).

MATERIALS AND METHODS

T4CSA was obtained from Eastman Kodak Ltd, and purified by repeated recrystallization from hot aqueous-ethanolic solution till a sharp melting point of 150–151°C was obtained (literature 149–151°C). Protamine sulphate was obtained from Sigma Co. Ltd, and used without further purification. The alcohols used (ethanol, propan-2-ol and ethylene glycol) were purified by refluxing under acid conditions with 2,4-dinitrophenylhydrazine. All other chemicals used were of 'Analar' grade.

The light source was a 220 W Hanovia UVS medium pressure lamp. This lamp has a strong emission line at 365 nm which can be isolated with a Chance OX1 filter. The filter has an absorbance of less than 0·5 in the wavelength range 330–390 nm with minimum absorbance at 365 nm. This 365 nm irradiation is strongly absorbed by the photochemically active anionic form I of T4CSA (Coxon *et al.*, 1965).

The reaction cell was cylindrical in shape (diameter 3·5 cms; height 6·5 cms) and constructed of pyrex glass. The volume of solution irradiated in each experiment was 25 ml. During irradiation the solution was continuously stirred by having a small magnetic follower encased in PTFE within the cell and this follower was activated by a rotating magnet beneath the cell. All irradiations were carried out with the reaction cell in a thermostatically controlled bath at 25°C.

Although earlier work (Jenkins *et al.*, 1964; Coxon *et al.*, 1965) had shown that chloride ions are released on photolysis of the anionic form of T4CSA in aqueous alcoholic solution, it was not specified whether the irradiated solutions were either aerobic or anaerobic. Our preliminary experiments showed that chloride ions are released under both conditions but more rapidly so when the solution is anaerobic. Before each irradiation, therefore, virtually all the oxygen was removed by presaturating the solution with nitrogen containing less than 5 p.p.m. of oxygen. Also, for those experiments carried out with the solution buffered at physiological pH ($\mathrm{pH}=7{\cdot}4$) the citric acid : disodium hydrogen phosphate mixture recommended by Hale (1965) was used.

The fluorescence measurements were made with a Baird-Atomic 'Fluorispec' spectrofluorimeter.

RESULTS AND DISCUSSION

Initially the photochemical experiments of Coxon *et al.* (1965) were repeated by Williams (1971) in these laboratories. 0·2% aqueous-alcoholic solutions of T4CSA were photolysed with 365 nm irradiation and the course of the reaction monitored by potentiometric titration of the chloride ions produced by the reaction (R = alkyl group):

$$\dot{\mathrm{C}}\mathrm{l}+\mathrm{RCH_2OH}\rightarrow\mathrm{Cl}^-+\mathrm{R\dot{C}HOH}+\mathrm{H}^+ \qquad (2)$$

In agreement with Coxon *et al.* (1965) it was found that with a solution of pH 11, for example, one chloride ion was liberated per molecule of T4CSA photolysed. Thus there is no reason to doubt the validity of reaction (1) as a primary step in the photochemistry of T4CSA and, in our view, as a possible process for initiating the short-term photobiological effect.

When, however, T4CSA was irradiated under the same experimental conditions but at a lower concentration (0·02%) it was found that one molecule of T4CSA is not only capable of yielding one chloride ion but can, in fact, yield up to three chloride ions depending upon the initial pH of the solution. It should be noted that the experiments of Coxon *et al.* (1965), Williams (1971) and the experimental data shown in Fig. 1 (a) were carried out with unbuffered solutions, the pH of the solution thus decreasing during the course of the reaction due to the liberation of protons by reaction (2).

Of more significance with regard to the mechanism of the long-term photobiological effect of T4CSA, we believe, are the results shown in Fig. 1 (b). In this experiment the conditions were the same as in Fig. 1 (a) but the solution was buffered at physiological pH. It is seen that on irradiation three chloride ions were liberated per molecule of T4CSA photolysed.

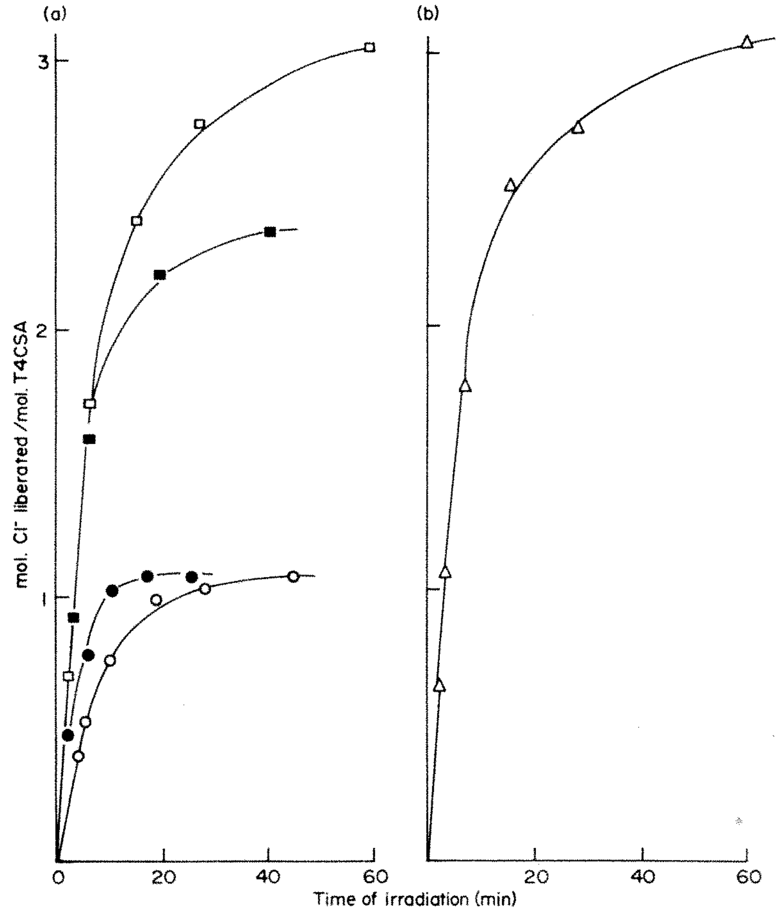

FIGURE 1. Photolysis of T4CSA in nitrogen-saturated 2:3 propan-2-ol/water solutions. (a) unbuffered solutions, initial pH = ○, 4·0; ●, 7·5; ■, 11·6 and □, 13·0. (b) buffered solution, pH = △, 7·4.

This interesting observation suggests the following mechanism for the long-term effect of T4CSA. If, following reaction (1), the radical II attaches itself to the protein moiety by means of a direct chemical bond then this could obviously increase the retention time of the compound in the protein. Also, as we have shown here, if the protein-attached trichloro-derivative is still potentially photochemically active then the long term photochemical sensitivity receives a logical explanation.

To test this theory the following experiment was carried out. T4CSA at a concentration of 2×10^{-4} mol/l was photolysed under neutral pH conditions in the presence of a simple protein-type compound, protamine sulphate (West *et al.*, 1966). The concentration of the protamine sulphate was 0·4% (by weight) and the solvent was a mixture of water and ethylene glycol, the protamine sulphate not being sufficiently soluble for the simpler alcohols to be used. The progress of the reaction was followed by spectrofluorimetry. This method was chosen because the photochemically active anionic form of T4CSA is quite strongly fluorescent with an emission maximum at about 450 nm. The corresponding T3CSA and T2CSA also fluoresce in the same wavelength region but more weakly so; T1CSA and salicylanilide itself are virtually non-fluorescent.

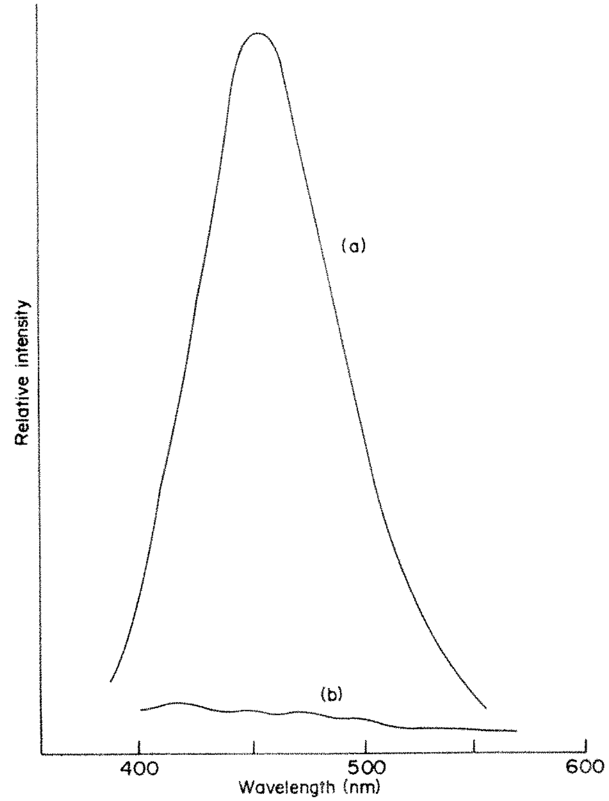

FIGURE 2. (a) Fluorescence from protamine-bound T4CSA formed after 15 min irradiation. (b) Absence of fluorescence after a further 30 min irradiation.

After irradiating for 15 min unbound T4CSA was removed by precipitating the protamine sulphate from solution with addition of propan-2-ol at −78°C to avoid denaturation. The mixture was then centrifuged. The precipitate was retained and the supernatant tested for fluorescence emission. This procedure was repeated several times until the supernatant showed no fluorescence emission indicating that all the chemically unbound T4CSA had been removed. The precipitated protamine sulphate containing the bound T4CSA was then redissolved in aqueous solution and its fluorescence emission is shown in Fig. 2. This result clearly indicates that radical-protein binding takes place on irradiation of T4CSA; a conclusion that is supported by the earlier experiments of Jenkins *et al.* (1964). When the solution was further irradiated for a period of 30 min it was found that all the fluorescence emission of the T4CSA bound complex had disappeared indicating photochemical conversion to the non-flourescent derivatives in agreement with the proposed mechanism.

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A study of physical light screening agents

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SUMMARY

In those photodermatoses in which the action spectrum involves wavelengths outside the short ultraviolet (i.e. >320 nm) little protection can be obtained against long UV (>320 nm) by the application of chemical light screening agents. An action spectrum involving wavelengths >320 nm is a common occurrence and may necessitate the additional provision of protection from a physical light screening agent. Such a physical agent is titanium dioxide and in a study of its use in a variety of formulations using *in vitro* techniques it was shown to be effective throughout the wavelengths 400–700 nm. It was also shown to have a satisfactory protective capacity in those photodermatoses in which the action spectrum involves wavelength peaks 365 nm (335–395 nm) and 400 nm (370–430 nm). It also has a reasonable cosmetic acceptability provided care is taken to incorporate in the formulations colouring agents which can be varied to suit the individual patient.

With the advent of equipment such as the diffraction grating monochromator (Sayre *et al.*, 1965; Berger, Urbach & Davies, 1967; Knox *et al.*, 1967; Cripps & Ramsay, 1970; Mackenzie & Frain-Bell, 1973) and its application to the study of action spectra in patients with photosensitivity eruptions, the choice of appropriate light screening agents has been put on a more satisfactory basis. It is now appreciated that the arbitrary choice of a light screening agent without first of all correlating its absorption spectrum with the action spectrum responsible for the skin reaction is a somewhat unrewarding procedure and has in the past been responsible for the incorrect assumption that light screening agents are of little use in the routine treatment of photodermatoses. If, for example, para-aminobenzoic acid or its ester amyldimethylaminobenzoate, which have absorption spectra mainly confined to the short UV sunburn wavelengths below 320 nm, are prescribed routinely for all cases of photodermatoses, they may well be ineffective or at best only produce partial protection. The reason is that the action spectra of these eruptions not infrequently involve long UV and visible wavelengths in addition to short sunburn UV or as in the case of porphyria are confined to wavelengths outside the long and short UV (>400 nm). In these conditions it is therefore necessary to use a protective agent capable of screening off the long UV and visible wavelengths, i.e. a physical and not a chemical barrier. The colour of any chemical agent capable of providing protection from these wavelengths would make it cosmetically unacceptable.

During the last few years we have investigated the effectiveness and cosmetic acceptability of titanium dioxide as a physical light screening agent in patients of both sexes suffering from a variety of photodermatoses and have found it effective and acceptable in many instances.

Titanium dioxide has been used in industry, e.g. in paint and ceramic manufacture, for years because of its light-scattering properties. It is also widely used in cosmetics; Calnan (1963) reported its use as a covering cream for skin blemishes and Smithers & Wood (1952) used it as a protective agent in the prophylaxis of xeroderma pigmentosum. We have previously drawn attention to its effectiveness as a light screening agent in patients in whom the action spectrum involved wavelengths >320 nm (MacLeod & Frain-Bell, 1971; Frain-Bell & Magnus, 1971). In these reports the preparation contained 20% titanium dioxide with other solid ingredients in a formulation which was not always cosmetically acceptable to male subjects. Since this report in 1971 it has been our objective to study further the use of titanium dioxide, to try to improve the formulation, and to increase as far as possible its cosmetic acceptability.

MATERIALS AND METHODS

The following ingredients were used:	Abbreviation
Titanium dioxide (British Titan, R-TC5)	—R-TiO_2
Titanium dioxide (B.D.H. Lab. Reagent)	—A-TiO_2
Red ferric oxide (Williams, Hounslow No. 171880)	—Fe_2O_3
Mexenone cream 4% (Ward Blenkinsop)	—Mex.Cr.
Mexenone cream base (Ward Blenkinsop)	—Mex.Cr. Base
Solution of burnt sugar B.P.C. 1959 (Evans)	—SBS
Zinc oxide B.P.	
Kaolin B.P.	
Ethanol B.P.	
Para-aminobenzoic acid (B.D.H. Lab. Reagent)	—PABA
Amyldimethylaminobenzoate (van Dyk—Escalol 506)	—Ester
Mexenone (Uvistat Powder—Ward Blenkinsop)	—Mexenone

They were used to produce the following physical barrier cream formulations (Nos. 1–13) using a spatula and slab, and compared later with the protective effect of two chemical barrier lotions (Nos. 14 and 15):

(1) R-TiO_2 5·0%
Fe_2O_3 0·5%
Mex.Cr. to 100%

(2) R-TiO_2 5·0%
Fe_2O_3 0·5%
Mex.Cr. Base to 100%

(3) A-TiO_2 5·0%
Fe_2O_3 0·5%
Mex.Cr. to 100%

(4) A-TiO_2 5·0%
Fe_2O_3 0·5%
Mex.Cr. Base to 100%

(5) R-TiO_2 5·0%
SBS 5·0%
Mex.Cr. to 100%

(6) R-TiO_2 5·0%
Fe_2O_3 0·5%
SBS 10·0%
Mex.Cr. to 100%

(7) R-TiO_2 5·0%
Mex.Cr. to 100%

(8) R-TiO_2 5·0%
Mex.Cr. Base to 100%

(9) A-TiO_2 5·0%
Mex.Cr. to 100%

(10) A-TiO_2 5·0%
Mex.Cr. Base to 100%

(11) Mex.Cr.

(12) Mex.Cr. Base

(13) R-TiO_2 20·0%
Zinc Oxide 6·0%
Kaolin 2·0%
Fe_2O_3 1·0%
Mex.Cr. to 100%

(14) PABA 5·0%
Ethanol 60·0%
Water to 100%

(15) PABA 5·0%
Ester 2·5%
Mexenone 0·5%
Ethanol 100%

The optimum particle size for light-scattering by titanium dioxide is about 0·2 μ and in a commercially available cosmetic grade such as the rutile form (R-TiO_2) the particle sizes are almost all between 0·25 and 1·0 μ. The laboratory reagent grade was the anatase form (A-TiO_2).

In vitro *study*

Thin films, 6 μ and 14 μ thick, were prepared between quartz surfaces using a 'plate and trough' method (MacLeod & Frain-Bell, 1971). The transmission and spectral diffuse reflectance curves of formulations 1–13 were obtained using a Unicam SP.8000 recording spectrophotometer with a diffuse reflectance attachment. (No correction was made for reflectance due to the quartz surfaces.) The diffuse transmission of barrier creams is complicated to define and measure in terms relevant to the efficacy of sunscreens, because scattering within a protective layer produces a diffuse irradiation of underlying tissues which depends upon the diffuseness of the incident radiation and upon the depth within the underlying tissue. Apparatus for diffuse transmission measurements (Walsh, 1958) was not available and so spectral transmission curves were obtained using the Unicam SP.8000 spectrophotometer normally as for non-diffusing samples. The transmissions, so measured, approximate

TABLE 1. Transmittance and reflectance measurements of 6 μ and 14 μ films between 400 and 700 nm

Formula	6 μ Film		14 μ Film	
	Average reflectance (%)	Average transmittance (%)	Average reflectance (%)	Average transmittance (%)
1	20	3	26	< 1 ?
2	18	4	23	3
3	20	4	21	2
4	20	9	24	4
5	25	1	27	< 1
6	22	3	21	< 1
7	27	2	29	< 1 ?
8	23	2	27	1
9	25	3	28	2
10	23	10	25	4
11	< 10 ?	25	< 10 ?	21
12	< 10 ?	50	< 10 ?	38
13	35	< 1	38	< 1

to direct transmission factors, since the photomultiplier detector is at a relatively large distance from the sample and detects only a small proportion of the diffusely transmitted radiation. Consequently, for the physical barrier formulations, the transmission factors as measured and quoted in Table I underestimate the total flux actually transmitted. Nevertheless these figures provide a useful guide to the relative merits to be expected of different physical barriers.

In vivo *study*

An assessment of the efficacy of formulae Nos. 1, 2, 6, and 13, containing variations of colouring agents (ferric oxide and burnt sugar solution) with or without mexenone, was carried out in a group of thirteen control subjects and in a group of sixteen subjects affected by a variety of photodermatoses. The degree of protection was compared with that obtained by using the chemical agents PABA, its ester, and mexenone (formulae Nos. 14 and 15).

In each subject the skin of the back was exposed to irradiation at wavelength peaks 305 nm (bandwidth 300–310 nm), 365 nm (bandwidth 335–395 nm) or at 400 nm (bandwidth 370–430 nm), a WG305 Schott filter being interposed between the light source and the skin at 365 nm peak wavelength, and a WG345 Schott filter at 400 nm peak wavelength so as to prevent exposure to scattered irradiation from other wavelengths. Prior to irradiation an area of 40 cm^2 was protected by the application of 100 mg of cream or 0·1 ml of lotion. Using a xenon arc diffraction grating monochromator system previously described (Mackenzie & Frain-Bell, 1973) multiples, e.g. ×2, 4, 6, and 8, of a previously defined minimal dose for erythema or erythema-papular responses were given and the protective factor determined, skin responses being recorded at 7 and 24 h after irradiation.

RESULTS

In vitro *study*

The reflectance measurements using the 6 μ films made with the plate and trough are listed in Table 1. The reproducibility of the films was within $\pm 0{\cdot}25\%$ reflectance. Percentage reflectance, to the nearest per cent at 400, 500, 600, and 700 nm, was averaged to produce the average reflectance over the range. Reflectance at 325 nm was $<10\%$ for all formulae 1–13. For the mexenone cream (formula 11) and the cream base without mexenone (formula 12) it was also $<10\%$ throughout most of the 400–700 nm range.

From the results in Table 1 it can be seen that the high concentrations of titanium dioxide (20%) and other solid ingredients in formula 13, the original cream ER1 (MacLeod & Frain-Bell, 1971), gave it the highest reflectance value of 35%. When the percentage of titanium dioxide was reduced to 5% as in formulae 7 and 9 the reflectance was reduced only to 27% (formula 7) and to 25% (formula 9) respectively. Equivalent formulations (8 and 10) which included only the cream base without mexenone had reflectance values of 23%, i.e. the presence of the mexenone had no effect on the efficacy of the formulation. The addition of the pigments (red ferric oxide and/or solution of burnt sugar) for cosmetic purposes as in formulae 1, 5 and 6 reduced the reflectance only marginally (formula 1 $=20\%$; $5=25\%$; and $6=22\%$) when compared with those formulations which contained no added pigments (formula $7=27\%$; $9=25\%$).

The reflectance values were similar, irrespective of the presence of the anatase titanium dioxide (A-TiO_2) with formulae 3 and 4 or of the fine particle size grade of titanium dioxide (R-TiO_2) in formulae 1 and 2.

When the films were increased in thickness from 6 μ to 14 μ, the reflectance values were increased within the range of 1% in formula 3 to a maximum of 6% with formula 1 (Table 1).

Figures for transmittance measurements of 6 μ films are presented in Table 1. Here the reproducibility of the results is within ±0·5%. Transmittance to the nearest per cent at 400, 500, 600 and 700 nm was averaged to produce the average transmittance over the range. Formula 13 (containing 20% titanium dioxide) was again best with transmittance of less than 1% over the entire wavelength range. The 5% titanium dioxide formulations (formulae Nos. 1–10) mainly transmitted from 1–4%, except formulae Nos. 4 and 10, in which the anatase grade (A-TiO_2) transmitted marginally more (9 and 10%) than the fine particle rutile grade (R-TiO_2), (formula 2=4% and formula 8=2%). On the other hand the mexenone cream (formula 11) and the cream base alone without the mexenone (formula 12) have much higher transmittance (formula 11=25% and formula 12=50%). The percentage transmittance of the 14 μ film was somewhat less than that for the 6 μ films.

In vivo *study*

In a group of thirteen normal subjects (Table 2) a comparison was made between the 5% (formula 1) and 20% (formula 13) titanium dioxide containing cream and between the cream base with (formula 1) and without (formula 2) mexenone. It was found that the protection afforded at wavelength peak 305 nm (bandwidth, 300–310 nm) by the 5% titanium dioxide was unaffected by the presence of the mexenone (protection factor (PF) 3·5 and 3·7) although the 20% titanium dioxide formulation raised the PF to 6.

TABLE 2. Protective factor of formulations Nos. 1, 2, and 13 at 305 nm (bandwidth, 300–310 nm) in thirteen normal subjects

Subject	Initials	Formula		
		1	2	13
1	C.K.	4·5	4·5	6·8
2	A.N.	4·5	1·5	8·3
3	S.W.	1·5	1·5	4·5
4	G.N.	1·5	4·5	4·5
5	J.M.	13·5	1·5	4·5
6	G.H.	1·5	1·5	7·5
7	P.B.	4·5	7·5	—
8	D.M.	4·5	1·5	—
9	J.A.	1·5	3·0	—
10	N.B.	3·0	5·0	—
11	M.F.	3·0	4·5	—
12	C.C.	2·0	5·0	—
13	G.R.	3·0	—	—
Mean protective factor		3·7	3·5	6·0

Table 3 contains the results of *in vivo* evaluations carried out at 365 nm peak (bandwidth 335–395 nm) in ten cases of photodermatoses. The 5% titanium dioxide (R-TiO_2) pigmented creams, formulae 1 and 6, with PF's of 5·7 and 6·4, were only slightly less effective than formula 13 (which contains 20% titanium dioxide), PF of 9·5; and all were much more efficient than the chemical barriers PABA (formula 14) and PABA+ester+mexenone (formula 15) with PF's of 3·1 and 3·0 respectively. The lack of protection obtained by the use of chemical barriers is even more pronounced at 400 nm peak as can be seen in Table 4 where the chemical formulae 14 and 15 gave virtually no protection and the physical barriers (formulae 1, 6, and 13) gave degrees of protection varying from 3·3 to 9·0.

TABLE 3. Protective factors of formulations Nos. 1, 6, 13, 14 and 15 at 365 nm peak (bandwidth, 335–395 nm)

Subject	Initials	Diagnosis	Formulations					Action spectrum of the eruption as defined when using—	
			1	6	13	14	15	Narrow waveband irradiations (½ bandwidths 5 nm)	Broad waveband irradiations (½ bandwidths 30 nm)
1	A.G.	P.L.E.	3·8	—	—	3·8	3·8	295–360	335–365
2	I.M.	P.C.T.	7·0	7·0	7·0	7·0	3·0	—	365–600
3	D.B.	P.D./A.R.	5·0	3·0	—	1·5	1·5	295–340	335–430
4	M.E.	P.D./A.R.	5·0	7·0	9·0	1·5	—	295–330	Normal
5	C.D.	D.L.E.	3·0	5·0	7·0	1·5	—	Normal	335
6	H.McB.	P.L.E.	3·0	3·0	5·0	1·5	—	Normal	Normal
7	S.McK.	P.L.E.	7·5	—	12·5	—	3·0	295–370	335–365
8	A.A.	P.D./A.R.	9·3	15·5	15·5	3·6	3·6	295–360	335–400
9	J.McF.	P.D./A.R.	1·7	1·7	9·0	3·6	—	250–325	335–400
10	I.W.	S.U.	11·3	11·3	11·3	4·3	—	250–330	335–500
Mean protective factor			5·7	6·4	9·5	3·1	3·0		

P.L.E., Polymorphic light eruption; P.C.T., Porphyria cutanea tarda; P.D./A.R., Photosensitivity dermatitis/actinic reticuloid; D.L.E., Discoid lupus erythematosus; S.U., Solar urticaria.

A field study of the protective action and cosmetic acceptability in a group of workers in a dye manufacturing process exposed to an anthraquinone based dye (Gardiner *et al.*, 1972), which produced a phototoxic skin reaction through wavelengths in the visible range (peak wavelength 600 nm), was conducted using formulae 5 and 6, each of which contains 5% titanium dioxide but with different colour ingredients. Out of a total of sixty-nine subjects using formula 5 and using a subjective assess-

TABLE 4. Protective factors of formulations Nos. 1, 6, 13, 14 and 15 at 400 nm peak (bandwidth, 370–430 nm)

Subject	Initials	Diagnosis	Formulations					Action spectrum of the eruption as defined when using—	
			1	6	13	14	15	Narrow waveband irradiations (½ bandwidths 5 nm)	Broad waveband irradiations (½ bandwidths 30 nm)
1	G.C.	P.D./A.R.	—	—	4·5	1·5	—	295–400	335–600
2	A.G.	P.C.T.	3·0	—	7·5	—	2·0	—	365–600
3	R.W.	P.L.E.	1·3	—	2·8	—	0·0	—	335–430
4	D.B.	P.C.T.	1·5	9·0	7·0	—	1·5	—	365–600
5	A.E.	P.C.T.	2·0	—	7·5	—	—	—	400–600
6	I.W.	S.U.	3·2	3·2	3·2	1·2	—	250–330	335–500
7	S.D.	E.P.P.	7·0	9·0	9·0	—	—	—	400–600
8	N.McD.	P.C.T.	7·0	7·0	9·0	—	—	—	400–600
9	D.B.	P.D./A.R.	1·5	—	2·5	—	0·0	290–340	335–430
Mean protective factor			3·3	9·0	6·0	1·4	0·9		

P.D./A.R., Photosensitivity dermatitis/actinic reticuloid; P.C.T., Porphyria cutanea tarda; P.L.E., Polymorphic light eruption; S.U., Solar urticaria; E.P.P., Erythropoietic protoporphyria.

ment only, forty-eight found it cosmetically acceptable when used on the face, sixty when used on the hands, and sixty-three obtained subjective and objective benefit. Of the fifty-two subjects who used formula 6, only twenty-four found it acceptable when applied to the face, forty-one when applied to the hands, and forty-five obtained subjective and objective reduction in the phototoxic skin response.

DISCUSSION

Titanium dioxide is a particularly useful agent for protecting the skin against the harmful effects of a wide band of solar radiation in patients with a variety of photodermatoses. This property is a function of its high refractive index (about 2·7) which gives it excellent light scattering properties. The difference between the refractive index of the titanium dioxide and its surrounding medium should be as great as possible to maintain this optical property. Also, the powder should be evenly dispersed in the cream base with no 'clumping' of the particles so as to retain maximum reflectance and absorbance.

The colouring agents (red ferric oxide and solution of burnt sugar) added for cosmetic purposes selectively absorb some of the light scattered by the titanium dioxide. These pigments allow for patient selection of a colour ranging from light flesh colour to light and dark tan. The addition of the colouring agent to formulae 1, 5, and 6, only marginally changes the optical properties of non-pigmented cream (formula 7) and the PF's of these creams are only slightly lower than the less cosmetically acceptable cream (formula 13).

It is evident from the results of this study that the chemical agents such as para-aminobenzoic acid are only of marginal benefit at wavelength peak 365 nm (335–395 nm) and of no value when reactions are produced at wavelengths around 400 nm peak (370–430 nm). Since the action spectrum in porphyria, photosensitivity dermatitis/actinic reticuloid, 'photosensitivity' drug reactions, photocontact dermatitis and in some cases of polymorphic light eruption, solar urticaria and summer prurigo involve wavelengths in the long UV and/or visible spectrum, a titanium dioxide physical barrier is essential either alone or in combination with the chemical agent. It has been our impression that these physical agents can be made cosmetically acceptable by the incorporation of suitable colouring agents.

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Immunoglobulins in the skin in dermatitis herpetiformis and their relevance in diagnosis

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SUMMARY

Eighty skin biopsies from fifty patients with dermatitis herpetiformis (DH) have been examined for immunoglobulin deposits by direct immunofluorescence. IgA was found in all fifty patients. However, in two patients no IgA was detected in their first biopsy, and it is stressed that if the clinical suspicion of DH is high and no IgA is found in a single biopsy, then the biopsy should be repeated.

There are two distinct patterns of immunoglobulin deposition in DH. The most common form of deposition is seen in the dermal papillae, termed the 'papillary' pattern. This pattern was the only one present in sixty-seven of the seventy-eight biopsies. A less common pattern is that of a 'continuous' line along the dermo-epidermal junction. This was the only pattern of immunoglobulin deposition in nine of the seventy-eight biopsies. In two biopsies both the papillary and continuous patterns were present.

IgA was found in all seventy-eight of the positive biopsies and was the only immunoglobulin detected in sixty-seven biopsies. In addition to IgA, IgM was present in seven biopsies, and IgG in two biopsies. In one biopsy IgM and IgG were present with the IgA.

The detection of IgA in the uninvolved skin in patients with DH is a simple test to perform, and at the present time is the most reliable way of establishing the diagnosis.

There is now considerable evidence to suggest that DH is due to an immunological abnormality associated with gluten sensitivity. As both the intestinal and skin lesions respond to gluten withdrawal (Fry *et al.*, 1968, 1969; Fry *et al.*, 1973; Barnetson, Heading & White, 1973), it has become important to diagnose DH with certainty so that specific treatment with a gluten-free diet (GFD) may be considered for a particular patient. The immunological features which have been described in DH include immunoglobulin deposition in the uninvolved skin (Cormane, 1967; Van der Meer, 1969; Chorzelski *et al.*, 1971; Seah *et al.*, 1972), the finding of complement in the uninvolved skin and early lesions (Chorzelski & Cormane, 1968; Van der Meer, 1969; Holubar, Doralt & Eggarth, 1971; Seah *et al.*, 1973a), the presence of anti-reticulin antibody in the serum and a high incidence of anti-nuclear antibodies (Seah *et al.*, 1971), and circulating immune complexes (Mowbray *et al.*, 1973). The immunoglobulin found in the skin is invariably IgA (Van der Meer, 1969, Chorzelski *et al.*, 1971; Seah *et al.*, 1971; 1972). Apart from the different class of immunoglobulin, two different patterns of deposition have been described. These are firstly, the 'granular' appearance found in the dermal papilla (Chorzelski *et al.*, 1971; Seah *et al.*, 1972; Fry & Seah, 1974) and secondly, a linear or continuous band described as being 'along' the basement membrane by Chorzelski *et al.* (1971) but considered by Seah *et al.* (1972) to be beneath the basement membrane. Because of the different classes of immunoglobulin involved and the different patterns there has been confusion over the interpretation of these immunofluorescence findings. Indeed some authors have re-introduced the argument, as has occurred in the past with the clinical and light microscopic findings, that there is an 'overlap' between DH and pemphigoid, based on the immunofluorescence findings (Honeyman *et al.*, 1972). We now report our immunofluorescence findings in fifty patients with DH and hope that the results and their interpretation will clarify the situation and enable the diagnosis of DH to be made with certainty on the basis of immunofluorescence studies.

PATIENTS AND METHODS

Patients

Fifty patients, twenty-eight males and twenty-two females whose ages ranged from 22 to 70 years, were studied. They had been referred to a special clinic for the investigation of dermatititis herpetiformis. All the patients had a history of an irritating eruption which was controlled by dapsone, and which relapsed on withdrawal of this drug. In addition, all the patients had IgA deposits in their uninvolved skin in at least one biopsy.

Skin biopsies. Eighty skin biopsies were examined. Thirty patients had a single biopsy of their uninvolved skin. Ten patients had two biopsies—five of these had two biopsies from uninvolved skin at different times, and in the other five, one biopsy from uninvolved skin and one biopsy from an early lesion at the same time. By an 'early lesion' we mean either a lesion where no obvious blister has formed, or the peribullous erythematous skin where a blister is present. Large, frank blisters, or older excoriated lesions were not examined. Ten patients had three biopsies. All of these ten had biopsies from an early lesion and two biopsies from uninvolved skin. One of the biopsies from the uninvolved skin was taken at the same time as the lesional biopsy, and the other at a different time. Skin biopsies were taken under local anaesthesia using lignocaine 1 or 2% and snap-frozen as previously described (Seah *et al.*, 1972). They were kept at −70°C until processed. The skin biopsies were processed as previously described (Seah *et al.*, 1972) for immunoglobulin deposits using monospecific fluorescein isothiocyanate (FITC) labelled anti-human IgG, IgM and IgA (Wellcome Reagents) at dilutions where minimal non-specific staining was seen. For immunofluorescence microscopy, a Reichert 'Diapan' microscope fitted with a quartz halogen light source, a FITC-3 'Balzer' primary interference filter and a Kodak Wratten 12 secondary filter were used.

Auto-antibody studies. Circulating anti-reticulin antibody was detected as described by Seah *et al.* (1971). Circulating antibodies to basement membrane (as found in bullous pemphigoid) and to intercellular cement substance (as found in pemphigus) were detected as described by Beutner, Jordan & Chorzelski, (1968) using guinea-pig lip and oesophagus as substrates. A polyvalent FITC-labelled sheep-anti-human immunoglobulin conjugate (Wellcome Reagents) was used at a dilution of 1:32.

RESULTS

Immunoglobulin class

Patients. IgA was present in all fifty patients studied (Table 1). IgM was present in addition to IgA in six patients, and IgG in addition to IgA in two patients. In one patient both IgM and IgG were found as well as IgA.

TABLE 1. Class of immunoglobulin

	IgA alone	IgA+IgM	IgA+IgG	IgA+IgM+IgG	No Ig.	Total
Patients	41	6	2	1	0	50
Biopsies	68	7	2	1	2	80

Biopsies. IgA was detected in seventy-eight of the eighty biopsies; IgM in eight, and IgG in three (Table 1). In the two biopsies in which no IgA was detected, neither IgG nor IgM was found. In six of the seven biopsies which showed the presence of IgM, and in two of the three biopsies with IgG, these immunoglobulins were found in addition to IgA. In one biopsy all three classes of immunoglobulin (IgA, IgG, IgM) were present. IgM or IgG were never detected as the sole class of immunoglobulin.

Pattern of immunoglobulin deposits

Two main patterns of immunofluorescent staining were seen in the skin biopsies. These have been previously described (Seah *et al.*, 1972) and are called the 'papillary' or the 'continuous' patterns.

Papillary only. In sixty of the seventy-eight positive biopsies immunoglobulins were present in the papillary form (Fig. 1a, b). In sixty of the sixty-seven biopsies IgA was present alone, in six IgM and IgA were present, and in one IgG and IgA (Table 2).

Continuous only. This was the pattern in nine of the biopsies (Fig. 2). In eight of the nine, IgA was the only class of immunoglobulin present, and in one IgG was found in addition to IgA (Table 2).

TABLE 2. Pattern of immunoglobulin deposits

	IgA alone	IgA+IgM	IgA+IgG	IgA+IgM+IgG	Total
Papillary alone	60	6	1	0	67
Continuous alone	8	0	1	0	9
Papillary and Continuous	0	1*	0	1†	2

* Papillary IgM, Continuous IgA; † Papillary IgA, Continuous IgM+IgG.

Papillary and continuous. In two biopsies, both the continuous and papillary patterns of immunoglobulin deposition were present. In addition, it was found that in both biopsies the class of immunoglobulin was different in the papillary and continuous deposits. In the one biopsy there was continuous IgA and papillary IgM, and in the other there was continuous IgM and IgG, and papillary IgA (Table 2).

Multiple biopsies of uninvolved skin at different times

Fifteen patients had two biopsies from uninvolved skin on two different occasions (Table 3). In nine of the fifteen patients the class of immunoglobulin, and pattern of immunofluorescence staining were the same. All nine of these patients had IgA deposits showing only the papillary pattern. Of the remaining six patients, two failed to show any immunoglobulin deposits on the first biopsy but showed

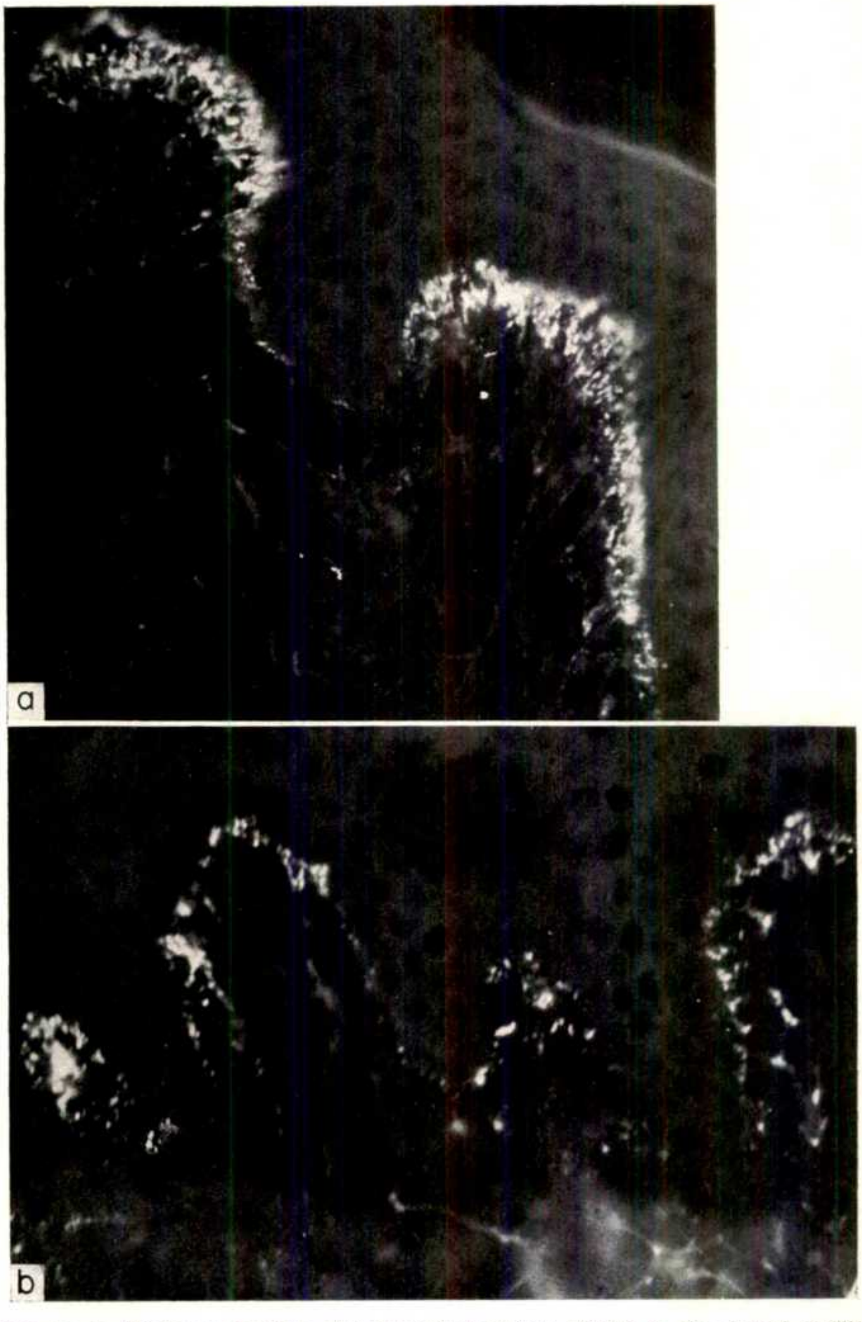

FIGURE 1a, b. High power views of 'papillary' deposition of IgA in the dermal papillae of uninvolved DH skin. Direct immunofluorescence—FITC-anti-human IgA (× 400).

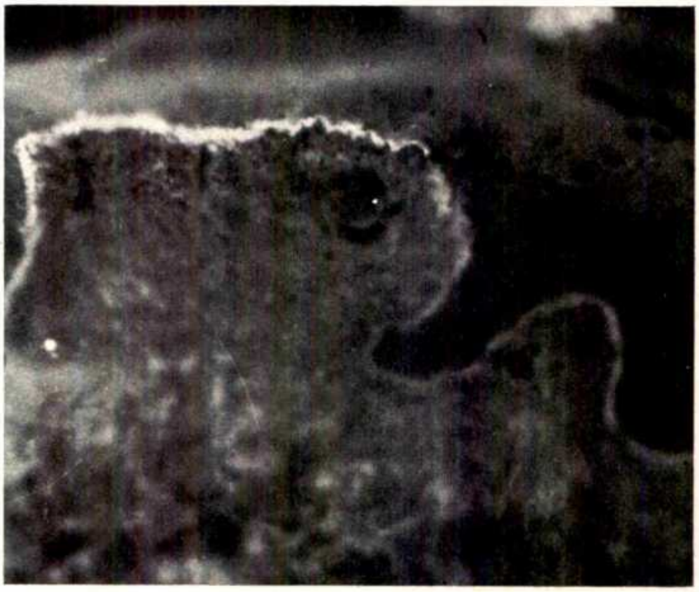

FIGURE 2. Uninvolved DH skin: Direct immunofluorescence using FITC-anti-human IgA showing the less common 'continuous' pattern of IgA deposition (× 160).

TABLE 3. Two biopsies from uninvolved skin at different times

Number of patients	1st Biopsy	2nd Biopsy
9	IgA (P)	IgA (P)
2	0	IgA (P)
1	IgG (C)+IgM (C) IgA (P)	IgA (P)
1	IgM (P)+IgA (P)	IgA (P)
1	IgM (P)+IgA (C)	IgA (C)
1	IgA (P)	IgM (P)+IgA (P)

P, Papillary pattern; C, Continuous pattern.

papillary IgA on the second biopsy. Two other patients showed papillary IgA alone on one biopsy and papillary IgA and IgM together on the other biopsy. The remaining two patients showed differences in both the class and pattern of immunoglobulin deposition. Thus, one patient showed papillary IgA and continuous IgG and IgM on the first biopsy, but only papillary IgA on the second biopsy. The other patient showed papillary IgM and continuous IgA on the first biopsy, but only continuous IgA on the second.

Comparison of uninvolved skin and early lesions

Immunoglobulin deposits were found in all the fifteen biopsies from early lesions. In fourteen of these fifteen patients, the class and pattern of immunoglobulin deposition was the same as that found in the uninvolved skin. In ten of the fifteen patients only IgA in a papillary pattern was found in both the uninvolved and early lesions (Table 4). In three other patients IgA in a continuous pattern was

TABLE 4. Comparison of immunoglobulin deposits in involved and uninvolved skin

Number of Patients	Uninvolved Skin	Early Lesions
10	IgA (P)	IgA (P)
3	IgA (C)	IgA (C)
1	IgA (P) IgM (P)	IgA (P) IgM (P)
1	IgA (P)	IgA (P) IgM (P)

P, Papillary pattern; C, Continuous pattern.

present in both involved and uninvolved skin. Of the remaining two patients, one had IgA and IgM in a papillary pattern in both the involved and uninvolved skin, and in the patient in whom there was a difference between involved and uninvolved skin, IgA in a papillary pattern was present in the uninvolved skin, whilst in the involved skin IgA and IgM were both present in a papillary pattern.

Pattern and class of immunoglobulin deposition in uninvolved skin in relation to treatment

In all there were sixty-five biopsies from the uninvolved skin. Of these, thirty-six biopsies were from patients taking dapsone, fourteen from patients whose eruption was controlled with a gluten-free diet (GFD), and fifteen from patients not receiving any therapy. There appeared to be no significant difference in the class or pattern of immunoglobulin deposition in relation to treatment. IgA alone in a papillary pattern was the most common finding in all three groups, being present in thirty of the thirty-six patients taking dapsone, ten of the fourteen patients taking a GFD, and ten of the fifteen patients not receiving any therapy (Table 5). IgA in association with IgG or IgM, or both, was present in two of the patients taking dapsone, four of the patients taking a GFD, and one of the patients not receiving any therapy. The numbers involved are too few to allow any statistical comparison. IgA in a continuous pattern was found in three patients taking dapsone, in one patient taking a GFD, and in three patients not receiving any therapy. Once again however, the numbers are too small for statistical analysis. It would appear however, that there is no correlation between the class and pattern of immunoglobulin deposition and therapy.

TABLE 5. Class and pattern of immunoglobulin deposits in the uninvolved skin in relation to treatment

Treatment	Pattern of Ig deposit	IgA	IgA+IgM	IgA+IgG	IgA+IgM+IgG
Dapsone	Papillary	30/36		Nil	Nil
	Continuous	2/36	1/36*	1/36	Nil
GFD	Papillary	10/14	2/14	1/14	
	Continuous	Nil	Nil	Nil	1/14†
Nil	Papillary	10/15	1/15	Nil	Nil
	Continuous	3/15	Nil	Nil	Nil

* Papillary IgM, Continuous IgA; † Papillary IgA, Continuous IgM+IgG.

Serological studies in patients with the continuous pattern of immunoglobulin deposition
In the seven patients who showed the continuous pattern of immunoglobulin deposition in one or more of their biopsies, anti-reticulin antibody was present in one. No circulating antibodies to basement membrane or epidermal intercellular cement substance were detected.

DISCUSSION

IgA in the uninvolved skin is now considered to be the most reliable criterion for the diagnosis of DH (Fry & Seah, 1974). In this study the pattern of IgA deposition and its possible association with IgM and/or IgG are described. It should be stressed that the uninvolved skin is the site to be studied for immunoglobulin deposits for diagnostic purposes. Thus, the original studies of Jordan *et al.* (1967) and Chorzelski & Cormane (1968) failed to reveal immunoglobulins in the skin in DH because actual lesions were investigated and not the uninvolved skin. It is now thought that the immunoglobulins in a lesion in DH are involved in the pathogenesis of the skin lesion (Seah *et al.*, 1973a), and are subsequently phagocytosed and destroyed by polymorphs attracted to the site by chemotactic agents.

Cormane (1967) was the first to describe the presence of immunoglobulins in the uninvolved skin in DH. The class of immunoglobulin however was not reported. In 1969, Van der Meer reported the presence of IgA in the uninvolved skin of ten out of twelve patients with DH. This finding of IgA in the uninvolved skin of DH has since been confirmed by Chorzelski *et al.* (1971), Holubar *et al.* (1971), Seah *et al.* (1972) and Van der Meer (1972). However, the incidence of IgA has not been 100% in all these reports as it is in this study. There appear to be two possible explanations for this. Firstly, it may be that not all patients included in these previous reports did in fact have DH. In Van der Meer's first report (1969) he found IgA to be present in all five patients with 'typical' DH, but only in five out of seven with 'atypical' DH. It is thus questionable as to whether all his patients with 'atypical' DH did in fact have the disease. The criteria for the diagnosis of DH have recently been reviewed by Fry & Seah (1974), and it is apparent that a number of patients diagnosed as having DH in the past, do not have the disorder. A second reason for the lower incidence of IgA positivity in the earlier reports is that a single section or even a single biopsy may be insufficient to exclude the presence of IgA. This fact is exemplified in the present study by the finding that in two of the fifty DH patients who had negative initial biopsies, examination of a second biopsy was positive for IgA. This same point is also stressed by Jablonska *et al.* (1973) who found that serial sections were necessary in three of their patients to detect IgA, and in one further patient a second biopsy was necessary after thirty consecutive sections of the first biopsy had failed to reveal IgA.

There appear to be two distinct patterns of IgA deposition in the uninvolved skin in DH. Firstly, in what we have termed the papillary pattern, immunoglobulins are found only in the dermal papilla. This pattern coincides with the granular, microgranular and fibrillar appearances of Chorzelski *et al.* (1971) and Van der Meer (1972). We feel that 'papillary' is a better term as it localizes the site of deposition of IgA, whereas the terms granular, microgranular and fibrillar refer solely to the appearance of the immunofluorescence seen on microscopy. Almost certainly the difference in appearance is simply due to whether the reticulin fibres where the IgA is deposited are sectioned transversely or longitudinally. The second pattern of immunoglobulin deposition seen in DH is the 'continuous' one. This has previously been reported by Chorzelski *et al.* (1971), but was not found by Holubar *et al.* (1971) or Van der Meer (1972). The continuous pattern follows the line of the basement membrane, but the immunoglobulins have been shown to be beneath the intact basement membrane by electron microscopic studies (Seah *et al.*, 1972). This is distinct from pemphigoid where the immunoglobulin deposits are on the basement membrane. The most common pattern of immunoglobulin deposition in DH is the papillary one. In this study it was present alone in sixty-seven of the seventy-eight biopsies (86%). In a further two biopsies it was present in addition to the continuous pattern of immunoglobulin deposition. The incidence of the continuous pattern of immunoglobulin deposition alone was nine out of seventy-eight (12%), but if taken together with the two biopsies in which it was present with the papillary pattern, it was present in eleven of the seventy-eight biopsies (14%). This is slightly lower than the incidence of Chorzelski *et al.* (1971) who found the continuous pattern in five of their nineteen patients.

In all seventy-eight biopsies in which immunoglobulins were detected, IgA was always present either as the papillary or continuous pattern. In seven biopsies IgM, and in two biopsies IgG were present in addition to the IgA. In one biopsy both IgM and IgG were present in addition to the IgA. Chorzelski *et al.* (1971) also reported IgM and IgG in addition to IgA in approximately a fifth of their nineteen patients, but Holubar *et al.* (1971) did not find IgM or IgG in any of their twelve patients. In our study, in the nine biopsies which had IgG or IgM in addition to IgA, the pattern of deposition of the two classes of immunoglobulins was the same in eight of the biopsies. In the one biopsy in which there was a difference, IgA was present in a continuous pattern and IgM in a papillary pattern. In the single biopsy in which all three classes of immunoglobulins were present, IgA was present in a papillary pattern, and IgG and IgM in a continuous pattern. In the fifteen patients who had two biopsies from the uninvolved skin at different times, six patients had different results. In two, the biopsy was repeated because no immunoglobulin was found in the first biopsy, but was in the second biopsy. In the remaining four, there was a difference in the class of immunoglobulins detected; in three of these patients there were also different patterns of immunoglobulin deposition between the first and second biopsies. It is not possible to explain these differences at the present time, but they did not appear to be related to a difference in the clinical features in the disease, age or sex of the patient, nor in the treatment at the time of biopsy. In addition, there was no difference in the class or pattern of immunoglobulin deposition between those patients whose rash was being controlled with dapsone, those being controlled by a GFD, and those not receiving any treatment.

Apart from in DH, immunoglobulin deposits in the skin have now been described in a number of disorders affecting this organ. These include pemphigus, pemphigoid, lupus erythematosus, cicatricial pemphigoid and more recently, lichen planus (Michel & Sy 1973, Baart de la Faille-Kuyper & Baart de la Faille, 1974). The site and pattern of deposition, and the class of immunoglobulin involved are now being increasingly used for diagnostic purposes and in helping to understand the pathogenesis of these diseases. We feel that a number of points about immunoglobulin deposition in the skin of patients with DH are now emerging which should be stressed to avoid confusion with other disorders. The papillary pattern of immunoglobulin deposition in the uninvolved skin appears to be distinct for DH and has not been described in other disorders at the present time. The continuous pattern, however, has given rise to confusion and to reports stating that it is difficult to distinguish pemphigoid from DH (Honeyman *et al.*, 1972). This confusion between pemphigoid and DH is understandable if only the pattern of immunoglobulin deposition is considered. However, if the class of immunoglobulin is considered, then in pemphigoid the immunoglobulin is either IgG or IgM (Sams & Jordan, 1973), whilst in DH it is IgA or IgA in addition to IgG or IgM. Further differentiation between the two diseases can be made by serological studies; in pemphigoid, 85% of patients are known to have circulating antibodies to the basement membrane, but this does not occur in DH. However, in the latter disease, 25% of patients have the anti-reticulin antibody in the serum (Seah *et al.*, 1973b). None of the seven patients in our study with 'continuous' immunofluorescence patterns had basement membrane antibodies, but one had anti-reticulin antibody.

A recent report by Holubar, Honigsman & Woolf (1973) of IgA in addition to IgG in a continuous pattern in two of five patients with cicatricial pemphigoid (CP) is of interest. A study by Bean & Michel (1973) of CP, however, showed only IgG to be present in five of their six patients, and this was in involved skin and mucous membrane, and it was present in only one of the six patients' uninvolved skin. In the study of Holubar *et al.* (1973) the biopsies were from involved or previously involved skin. It is reasonable that in diseases affecting the mucous membrane, as in CP, IgA should be present as part of the pathology, for IgA is the main immunoglobulin involved in the immune response of mucosal surfaces, including the buccal cavity and the gastrointestinal tract. A corollary to this is the finding of IgA in the skin in DH—a disease in which small intestinal disease is known to exist (Marks, Shuster & Watson, 1966; Fry *et al.*, 1967). If this is the case, then IgA in a continuous pattern cannot be considered to be distinctive for DH. Electron microscopic studies, however, may well be able to differentiate between the two conditions by localizing the site of immunoglobulin deposition, i.e., whether it is below the basement membrane in DH, or on it as in CP. It is just possible that the two patients that Holubar *et al.* (1973) described with IgA and IgG in the skin had DH in addition to, or instead of CP, but no studies relating to the small intestine or response to dapsone were given in their report. We ourselves have one patient who has both CP and DH, indicating that the two diseases can occur together.

In both lupus erythematosus and lichen planus the immunoglobulin deposits occuring in the region of the basement membrane are not of the IgA class (Jablonska *et al.*, 1973; Baart de la Faille-Kuyper & Baart de la Faille, 1974), and therefore do not give rise to confusion with DH. It should be pointed out however that there are a number of reports of 'globules' of IgA in the skin. These deposits were described by Chorzelski *et al.* (1971) and Seah *et al.* (1972), and are found in normal skin and are probably not indicative of any pathology. The significance and nature of these deposits remain undetermined.

The presence of IgA in the skin, especially the clinically uninvolved skin, is a useful marker in the diagnosis of DH. In our series, it was found in all fifty patients studied, and the point has been made that the diagnosis of DH should not be considered in its absence (Fry & Seah, 1974). The significance of these deposits, however, remains a mystery although it is likely that they play some role in the pathogenesis of the skin lesions of DH.

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Contact dermatitis in animal feed mill workers

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SUMMARY

Three cases of contact dermatitis due to additives in animal feed substances are described occurring in animal feed workers. There were two cases of sensitivity to ethoxyquin and one to halquinol.

The processing of modern animal feeds has become a complex process requiring the addition of vitamins, hormones, antibiotics, antioxidants and various other chemicals. It is often not apparent to those working with these substances, or to the doctor with a patient from such a factory, which chemicals are being handled. Some chemicals are present in very minute quantities in the final product and may not be thought significant enough by the manufacturers to inform a dermatologist looking for the causes of a contact allergy. Contact dermatitis in workers making animal feeds has not often been reported. Most cases of contact dermatitis due to animal feeds have been in those actually feeding animals (Melhorn & Beetz, 1971; Neldner, 1972), though recently Wahlberg (1974) has found three cases of ethoxyquin sensitivity in animal feed workers. A rather similar position occurs with cement, in that dermatitis occurs commonly in those at the end point rather than those engaged in manufacturing. In a recent outbreak of contact dermatitis due to quinoxaline dioxide, most cases were farmers (Scott & Dawson, 1974).

CASE REPORTS

Case 1

The patient, a 30-year-old male, had worked in an animal feed mill for 5 years. His job was to add various supplements to the meal. This he did by emptying various packets of chemicals down a shute. He developed a generalized dermatitis, worst on the face and arms. This cleared on going off work and reappeared on return to work. Patch tests with animal feeds, including some supplemented meals, applied neat were positive. Subsequent patch tests with vitamin additives gave positive reactions with A and D_3 supplements. This suggested that the dermatitis was being caused by some common factor in these additives. These vitamins are dispersed in a solid gelatine matrix to convert them to a free flowing powder. Small traces of starch are added in the drying process. With persistent enquiry from the vitamin manufacturer, it was found that ethoxyquin was added as an antioxidant. Patch tests with the vitamins, gelatine and starch, were negative but 0·5% ethoxyquin in vaseline gave a strong positive reaction.

He now works as a gate-man in the factory and his skin is clear.

Case 2

This patient was a 48-year-old foreman in an animal feed mill. The rash, which began on the palms, spread to involve all parts of both hands and the soles and dorsa of the feet; it had been present for 2 years when first seen.

It had cleared completely on several occasions when he was off work. He was patch tested with the finished meal, and gave a positive reaction to a number of different meals. He was then patch tested with 0·5% ethoxyquin in soft white vaseline, giving a very marked reaction.

Since changing to work which does not involve contact with animal feeds his skin has remained clear.

Case 3

The patient, aged 42 years, had worked for 29 years in an animal feed mill as a foreman. Two weeks prior to the onset of the rash he had been assisting in adding 'Quixalaud' to the feeds. A contact dermatitis developed on the backs of the hands and wrists. The rash cleared up on going off work and did not recur on going back to work which avoided contact with Quixalaud. A patch test with 1% Quixalaud (halquinol 60%, chalk 40%) gave a marked positive reaction.

DISCUSSION

The purpose of this report is to draw attention to two additives in animal feeds which can cause contact dermatitis. It is probable that dermatitis from the cereal used in meal seldom occurs and that if looked for, most cases of contact dermatitis to animal feeds are due to some additive. It is difficult to get exact information about constituents of additives, often because it is not considered relevant or because the substance is thought to be present in such small quantities. Such was the case with ethoxyquin. Vitamins A and D_3 as supplied by the manufacturer contain 5% ethoxyquin (6-ethoxy-1, 2-dihydro-2, 2, 4-trimethylquinoline) as an antioxidant preservative. These vitamins are usually diluted with cereal in a premix which dilutes the ethoxyquin to between 0·2% to 0·15%. Further dilution in the final meal would end with a finished product containing 0·0001% ethoxyquin from the vitamin sources. However, it should be remembered that ethoxyquin also appears in the meal from two other sources. Fish and bone meal and tallow have an antioxidant, which is likely to be ethoxyquin, added at source and further ethoxyquin is added to the meal as a preservative before dispatch so that the meal ends up with a concentration of 0·01% ethoxyquin. Though attention was drawn to this sensitizer through its presence in vitamin additives where it is likely to be met in highest concentration, it is clearly a risk anywhere in an animal feed firm.

Ethoxyquin can be widely used in the food preparation industry in canned food, frozen foods, sauces, salad creams etc, and it would be surprising if more cases did not occur than is generally believed. These unrelated sources could also be a cause of flare-up in a sensitized person. Other antioxidants can be used in animal feed and butylated hydroxytoluene (Topanol) is probably more widely used than ethoxyquin. Wood & Fulton (1972) found ethoxyquin dermatitis a common condition in apple tickers in British Columbia. It is added to the apples in 0·3% aqueous solution to prevent 'scald' during storage and they have had twenty-five cases. A case of ethoxyquin dermatitis has been described in a fodder doser by Melhorn & Beetz (1971), and three cases in animal feed workers by Wahlberg (1974). Schubert, Goring & Gans (1973) have also described cases of ethoxquin dermatitis in feedstuff workers.

The concentration of ethoxyquin used for patch testing was 0·5% because this was the concentration used by Woods & Fulton. Melhorn & Beetz (1971) used 0·2% and 1%. Dawson (1974) has patch tested twenty control men who use animal feeds, with 1% ethoxyquin with no positive results. So piseems that 0·5% can be accepted as showing a true allergic response.

Halquinol is added to feeds for prevention and treatment of scours in pigs caused or complicated by *E. coli* and *Salmonella* spp. and those adding it are exposed to a high concentration. It is a chlorinated derivative of 8-hydroxyquinaline (oxine) consisting of the following three constituents, added in a 0·1% concentration to meal:

5, 7-dichloro-8-hydroxyquinoline; 5-chloro-8-hydroxyquinoline;
7-chloro-8-hydroxyquinoline.

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APPENDIX

It was thought helpful to include a list of chemicals which are liable to be added to animal feeds.

Antioxidants
Octyl gallate—Octyl 3, 4, 5,-trihydroxybenzoate; Dodecyl gallate—Dodecyl 3, 4, 5,-trihydroxybenzoate; N-propyl gallate—N-propyl 3, 4, 5,-trihydroxybenzoate; BHA—mixture of 3-and 2-*tert.*butyl 4-hydroxyanisole; BHT-2, 6-di*tert.*butyl-4-methylphenol; Ethoxyquin—6-ethoxy-1, 2-dihydro-2, 2, 4-trimethylquinoline.

Colourants
Capsanthen; Lycopen; Beta-8-apo-carotenal; Ethyl ester of beta-8-apo-carotenoic acid; Lutein; Cryptoxanthin; Violaxanthin; Canthaxanthin; Zeaxanthin; Patent Blue V; Curcumin; Amaranth; Tartrazine; Orange G; Green S; Indigo carmine; Brilliant Black (Black PN); Carmoisine; Ponceau 4R; Sunset Yellow FCF; Brown FK; Red 6B.

Vitamins
Various.

Trace elements
Iron; Iodine; Cobalt; Manganese; Zinc; Molybdenum; Selenium.

Emulsifiers, stabilizers and binders

Lecithins; Alginic acid; Sodium alginate; Potassium alginate; Ammonium alginate; Calcium alginate; 1,2-dihydroxypropyl alginate; Agar–agar; Carrageen; Carob seed flour; Tamarind seed flour; Guar seed flour, guar gum; Gum tragacanth; Gum arabic; Sorbitol; Mannitol; Glycerol; Pectins; Methylcellulose; Carboxymethylcellulose; Hydroxypropylmethylcellulose; Ethylmethylcellulose; Sodium, potassium or calcium salts of food fatty acids derived either from edible oils and fats, or from distilled food fatty acids; Mono- and di-glycerides of food fatty acids; Mono- and di-glycerides of food fatty acids esterified with the following acids:

(a) acetic, (b) lactic, (c) citric, (d) tartaric, (e) mono-acetyltartaric and di-acetyltartaric.

Sucrose esters—esters of sucrose and of food fatty acids; Sucro glycerides—esters of sucrose and of mono- and di-glycerides of food fatty acids; Polyglycerol esters of the non-polymerized fatty acids; Propylene glycol (1, 2-propanediol) esters of the food fatty acids; Sodium stearoyl-2-lactylate; Calcium stearoyl-2-lactylate; Lignosulphonates; Kaolin; Bentonite and other montmorillonite clays; Silica and silicates; Gelatine; Sodium hexametaphosphate; Sorbitan esters; Polyoxyethylene sorbitan esters; Disodium ethylenediamine tetra-acetate; Vermiculite; Esters of polyethylene glycol.

Other additives, antibiotics etc.

Copper Sulphate; Copper Oxide; Progen Arsanilic Acid; 3-Nitro-4-Hydroxphenyl-arsonic acid; Payzone (Nitrovin); Dumax Zinc Bacitracin; Maxymin (Dimethylstilboestrol and Methyltestosterone); Flavomycin Premix (Flavomycin); Eskalin (Virginiamycin); Coyden (Clopidol); D.O.T. (Dinitro-o-toluamide); Zoamix (Dinitro-o-toluamide); Embazin (Sulphaquinoxaline); Deccox (Decoquinate); Emtryl Premix (Dimetridazole); Pancoxin (Amprolium–Sulphaquinoxaline–Ethopabate); Supacox (Amprolium–Sulphaquinoxaline–Ethopabate–Pyrimethamine); Amprolmix UK (Amprolium–Ethopabate); Hygramix (Hygromycin B); Neftin Supplement (Furazolidone); Neftin Premix (Furazolidone); Bifuran Supplement (Nitrofurazone, Furazolidone); Salfuride (Nifursol); Terramycin TM (Oxytetracycline HCl); Sermix (Reserpine); Tylan AF (Tylosin Phosphate); Aurofac (Aureomycin–Chlortetracycline HCl); Cyfac (Aureomycin–Chlortetracycline HCl–Sulphadimidine–Procaine Penicillin).

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Trichophyton rubrum infections according to age, anatomical distribution and sex

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SUMMARY

Trichophyton rubrum caused 79·2% of the cases of dermatophytosis observed in a sample of caucasoids from Philadelphia. Tinea pedis was found in 84% of the female and male patients with dermatophytosis. Tinea manuum occurred with equal frequency in both sexes whereas tinea unguium was more prevalent in females. Tinea cruris occurred almost exclusively in males. Infections of two or more anatomical sites were observed more frequently in males. The data are compared with those reported by Rosman (1966) from a similar study done in Copenhagen.

Trichophyton rubrum infections have become more prevalent in many countries. Additional data are necessary in order to evaluate the course of these infections and to compare the clinical manifestations in different countries. Rosman (1966) carefully analyzed 240 cases seen in Copenhagen from 1961 to 1964. The present paper examines 232 cases of *T. rubrum* infections seen in Philadelphia from 1969 to 1971. The cases were categorized according to age, anatomical distribution and sex. All patients in the present study are caucasoids which, therefore, permits a comparison between the Copenhagen and Philadelphia studies.

MATERIALS AND METHODS

Materials and methods used in collecting the data have been reported previously (Blank, Mann & Reale, 1974). *T. rubrum* infections accounted for 79·2% of the 293 female and male caucasoid patients with dermatophytosis.

In the present study, the differences between means were evaluated by Fisher t tests and differences between proportions were compared by χ^2 tests; in either test, differences with a probability of occurrence due to chance of less than 0·05 were considered significant.

RESULTS

Table 1 shows the distribution of our cases according to age and sex. Infections occurred in all age groups; however, few infections were found in patients below 10 years of age. The average age of the

TABLE 1. Distribution according to age and sex

Years of age	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70+	Total
Females	—	8	3	6	14	17	6	5	59
Males	3	14	25	40	43	24	15	9	173
Total	3	22	28	46	57	41	21	14	232

females was 46·1 ± (s.e.) 2·2 which was significantly higher (t=2·11, d.f. 230; P<0·05) than in males (40·7 ± 1·2)

The frequency of infections was significantly higher in males (74·6%) than in females.

The anatomical distribution of lesions in females and males is presented in Table 2. Tinea pedis was observed in 84% of the female and male patients. Infections of the genital region and buttocks were almost exclusively restricted to males. The frequencies of hand, trunk, forearm, neck and face infections were similar in females and males. The frequency of lower leg infections was significantly higher in females than in males. Among patients with lower leg infections, folliculitis was more common in females (41·2%) than in males (10%).

TABLE 2. Location of infections

Site	Female No. (%)	Male No. (%)
Feet	50 (84·7)	146 (84·4)
Genitals	2 (3·4)	82 (47·4)
Buttocks	2 (3·4)	37 (21·4)
Hands	18 (30·5)	51 (29·5)
Lower Legs	(1) 17 (28·8)	(2) 30 (17·3)
Trunk	8 (13·6)	20 (11·6)
Forearms	7 (11·9)	15 (8·7)
Neck	3 (5·1)	9 (5·2)
Face	3 (5·1)	4 (2·3)

Number of patients with folliculitis: (1) seven; (2) three.

The frequencies of feet and hand infections are presented in Table 3. Tinea pedis generally involved skin and nails, especially in females (76%). Infections of the hands showed a different pattern; nails were involved in 83·3% of the females and 52·9% of the males.

Table 4 presents the number of toenail infections according to age and sex. These infections occurred more frequently with increasing age in both sexes and significantly more often in females than in males.

The anatomical sites of infection are listed in Table 5. *T. rubrum* infections were not found on the scalp. The frequency of two or more infected sites was lower in females than in males.

TABLE 3. Infections of feet and hands

	Skin only No. (%)	Nails only No. (%)	Skin and Nails No. (%)
Feet			
Females	8 (16·0)	4 (8·0)	38 (76·0)
Males	53 (36·3)	4 (2·7)	89 (61·0)
Hands			
Females	3 (16·7)	11 (61·1)	4 (22·2)
Males	24 (47·1)	10 (19·6)	17 (33·3)

TABLE 4. Toenail infections according to age

Age (years)	0–39 No. (%)	40+ No. (%)	Total No. (%)
Females	10 (58·8)	32 (76·2)	42 (71·2)
Males	30 (36·6)	63 (69·2)	93 (53·8)

TABLE 5. Sites of infections

One region affected	Female No.	(%)	Male No.	(%)
Feet	23	(39·0)	50	(28·9)
Genital	—	—	9	(5·2)
Legs	2	(3·4)	2	(1·2)
Hands	1	(1·7)	1	(0·6)
Trunk	1	(1·7)	1	(0·6)
Total	27	(45·8)	63	(36·5)
Two or more regions affected	32	(54·2)	110	(63·5)

DISCUSSION

The data in the present study and those from Copenhagen (Rosman, 1966) are comparable. *T. rubrum* caused 40% of the dermatophyte infections in Copenhagen which was lower than the 79·2% observed in our study. In both investigations, the male patients predominated (Copenhagen, 65%; Philadelphia, 75%) and few infections were found in patients under 10 years old. In Copenhagen, the frequency of infections was highest in male patients below the age of 39 years whereas in Philadelphia more males than females were infected in all age groups.

Tinea pedis occurred with the same frequency in both studies regardless of sex. Tinea pedis appears to be the 'first stigma of more widespread infection' (Rosman, 1966). As in Copenhagen, the incidence of toenail infections increased with age and was higher in females. Granulomatous folliculitis of the lower legs, first described by Cremer (1953), was diagnosed by Rosman (1966) in females only, whereas three cases were encountered in the males in our study.

The major difference between the Copenhagen and Philadelphia studies was in the frequency of patients with infections occurring at two or more body sites. Approximately 30% of the patients seen by Rosman (1966) had lesions at multiple anatomical sites whereas 54·2% of our female and 63·5% of our male patients had multiple sites of infection. Therefore, our patients had higher frequencies of infections of the lower legs, trunk, forearms and hands than the patients seen in Copenhagen.

Analyses of comparable data from other countries should lead to further insights into the different aspects of dermatophytosis so frequently caused by *T. rubrum.*

ACKNOWLEDGMENT

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A search for microtubular inclusions in endothelial cells in a variety of skin diseases

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SUMMARY

An electron microscopic study of a variety of skin diseases has demonstrated microtubular 'inclusions' in vascular endothelium in bullous pemphigoid for the first time. The 'inclusions' are similar to those originally reported in lupus erythematosus by Fresco (1968) and presently being detected in an increasing number and variety of pathological processes.

MATERIALS AND METHODS

Biopsies of the skin lesions (Table 1) were always carried out by an experienced dermatologist. The major portion was processed for conventional light microscopic examination. For all diseases an optimally disease-involved (as judged by the dermatologist) portion of skin (epidermis and dermis) was processed for electron microscopy thus: the tissue was reduced to cubic millimetre blocks within ice-cold, phosphate-buffered 2% glutaraldehyde and fixation continued for 2 h; the tissue was then washed with phosphate buffer, osmicated (1% phosphate-buffered osmium tetroxide) for 1 h, dehydrated and embedded in Araldite; sections were cut with an LKB ultramicrotome, double-stained with uranyl acetate and lead citrate and examined in a Siemen's 1A electron microscope.

RESULTS

Endothelial cell 'inclusions' were found (Tables 1 and 2) in four cases of systemic lupus erythematosus (SLE), one case of chronic discoid lupus erythematosus (CDLE), one case of poikiloderma associated with bronchial carcinoma and one case of pemphigoid. These structures (Table 2) were located within the cytoplasm of endothelial cells of capillaries (Fig. 1) and, in addition, within the endothelium of venules (2 cases of SLE) and the endothelium of one arteriole (case of SLE). 'Inclusion' concentrations varied from case to case (Table 2), being maximal in a case of SLE and minimal in another SLE case. As judged per section of cell, the highest population density of 'inclusions' was four in a case of SLE. The 'inclusion' complex was in every case located inside dilated segments of rough endoplasmic reticulum (RER) (Figs. 1 and 2) and less frequently within the perinuclear space (Fig. 3). In five

TABLE 1. Human skin biopsies studied

Biopsies	No. of cases
Systemic lupus erythematosus (SLE)	8
Chronic discoid lupus erythematosus (CDLE)	3
Bullous Pemphigoid	11
Poikiloderma with light sensitization	1
Scabies	1
Folliculitis decalvans	1
Bowen's disease	1
Erythema multiforme	1
Dermatitis herpetiformis	1
Rosacea	1
Mycosis fungoides	2
Lichen planus	1
Normal controls	4
Total	36

instances (Table 2) 'inclusions' appeared to lie free in the cytoplasm. The 'inclusion' was microtubular, being either a single highly convoluted unit or a large number of individual shorter intertwined units. The average diameter of the microtubule was 16 nm (range 12–21 nm) and the maximum dimension of a sectioned 'inclusion' in its entirety was 1·9 μm. No 'inclusions' were found after comparably detailed examination of four other cases of SLE, two other cases of CDLE, ten other cases of pemphigoid, one case of rosacea, two cases of mycosis fungoides, one case of scabies, one case of folliculitis decalvans, one case of Bowen's disease, one case of lichen planus, one case of erythema multiforme, one case of dermatitis herpetiformis and four samples of normal control skin.

DISCUSSION

This morphological investigation adds bullous pemphigoid to the existing list (*vide infra*) of 'inclusion'-positive diseases. The finding however throws no further light upon either the nature of the 'inclusion' or its basic relationship to the associated diseases. At the present time, a virus nature appears to be unlikely and this has been the finding in both non-neoplastic 'inclusion'-positive processes (Pincus *et al.*, 1970), and neoplastic 'inclusion'-positive states (Jenson *et al.*, 1971). However, Klippel, Grimley & Decker (1974) have raised the possibility of its being a transmissible agent (either viral or humoral) by their demonstration of the transient presence of identical 'inclusions' within the endoplasmic reticulum of neonatal umbilical cord blood lymphocytes in cases where the mothers had systemic lupus erythematosus.

Uzman *et al.* (1971) have made a list of 'inclusion'-positive cells, tissues, animals and disease processes. In the human, it has been recorded in endothelial cells, fibroblasts, glial cells, lymphocytes, monocytes, macrophages, mesenchymal tumour cells, cultured tumour cells, and cultured leukocytes. 'Inclusion'-positive tissues are skin, kidney, muscle, heart, synovium, brain, lymph node, blood leukocytes, spleen, thymus and a variety of tumours. 'Inclusion'-positive diseases are, (1) the 'connective tissue disorders', (2) a wide variety of kidney diseases, (3) neoplastic and reactive processes of the reticulo-endothelial system, (4) mesenchymal tumours, (5) a few epithelial tumours, (6) myocarditis, (7) mumps, (8) Krabbe leukodystrophy and (9) hepatitis. Identical structures have been identified in rabbit, pig, monkey, mink, horse, dog and hamster in diseases such as herpes simplex, TABLE 2. ‘Inclusion’-positive skin biopsies

Diseases	Vessel type affected	Cell affected	Population density of ‘inclusion’ per section of cell	Free and/or organelle-related	Largest dimension of whole ‘inclusion’ complex (μm)	Substructure	Diameter of microtubule (nm)
SLE	Capillary and venule	Endothelial	2	Within dilated RER	0·7	Microtubular Coiled Dense-packing	18
SLE	Capillary and venule	Endothelial	4	Within dilated RER and apparently free	1·7	Microtubular Coiled Dense-packing	21
SLE	Capillary and arteriole	Endothelial	3	Within dilated RER, perinuclear space, apparently free	1·9	Microtubular Coiled Dense-packing	15
SLE	Capillary	Endothelial	1	Within dilated RER, perinuclear space, apparently free	0·5	Microtubular Coiled Dense-Packing	12
Pemphigoid	Capillary	Endothelial	1	Within dilated RER	0·5	Microtubular Coiled Dense-packing	12
Poikiloderma	Capillary	Endothelial	2	Within dilated RER, perinuclear space, apparently free	1·0	Microtubular Coiled Dense-packing	16
CDLE	Capillary	Endothelial	2	Within dilated RER, apparently free	1·5	Microtubular Coiled Dense-packing	17

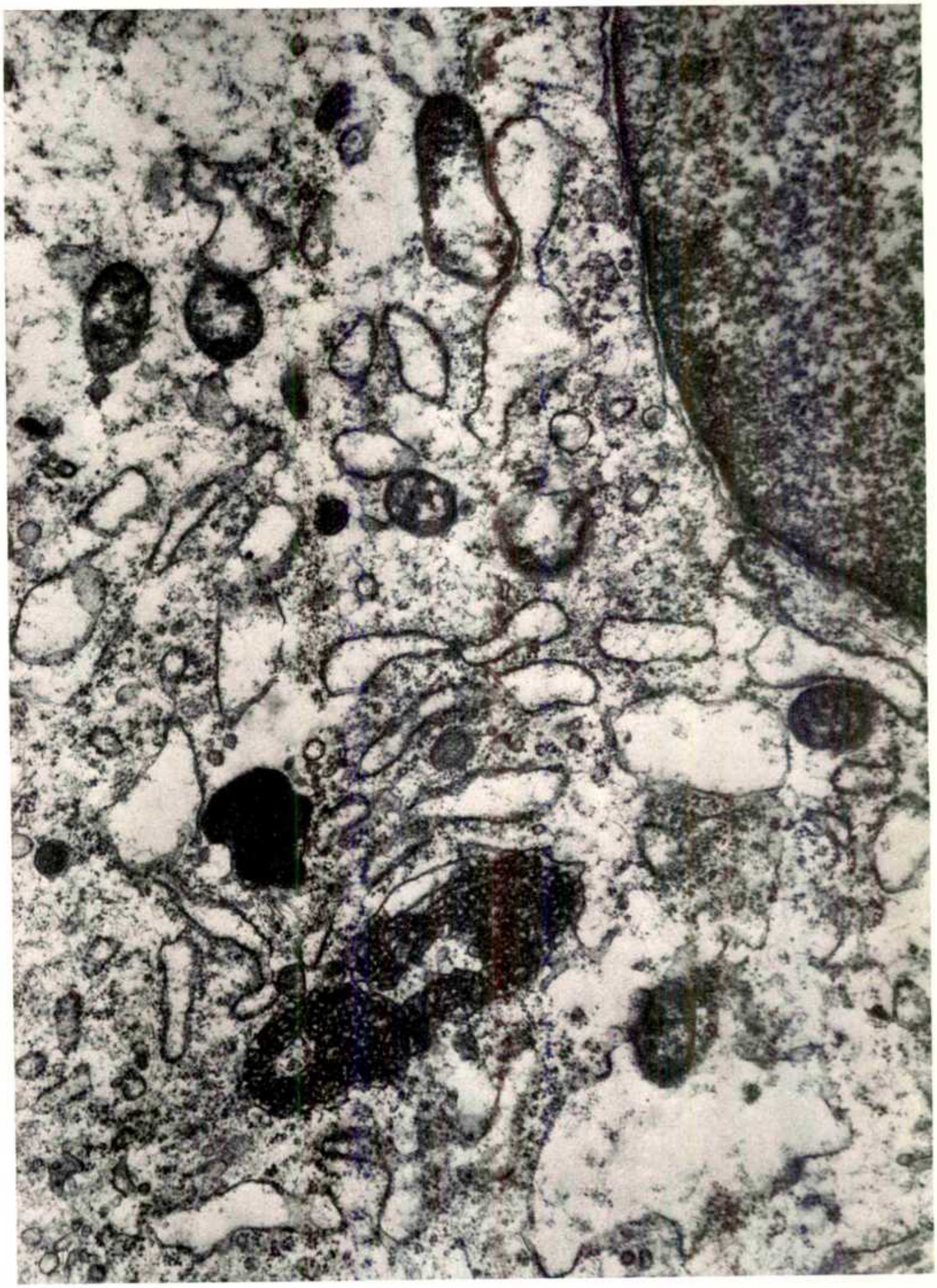

FIGURE 1. Electron micrograph of portion of endothelial cell showing the microtubular 'inclusion' within a dilated segment of the rough endoplasmic reticulum (uranyl acetate and lead citrate, × 32,400).

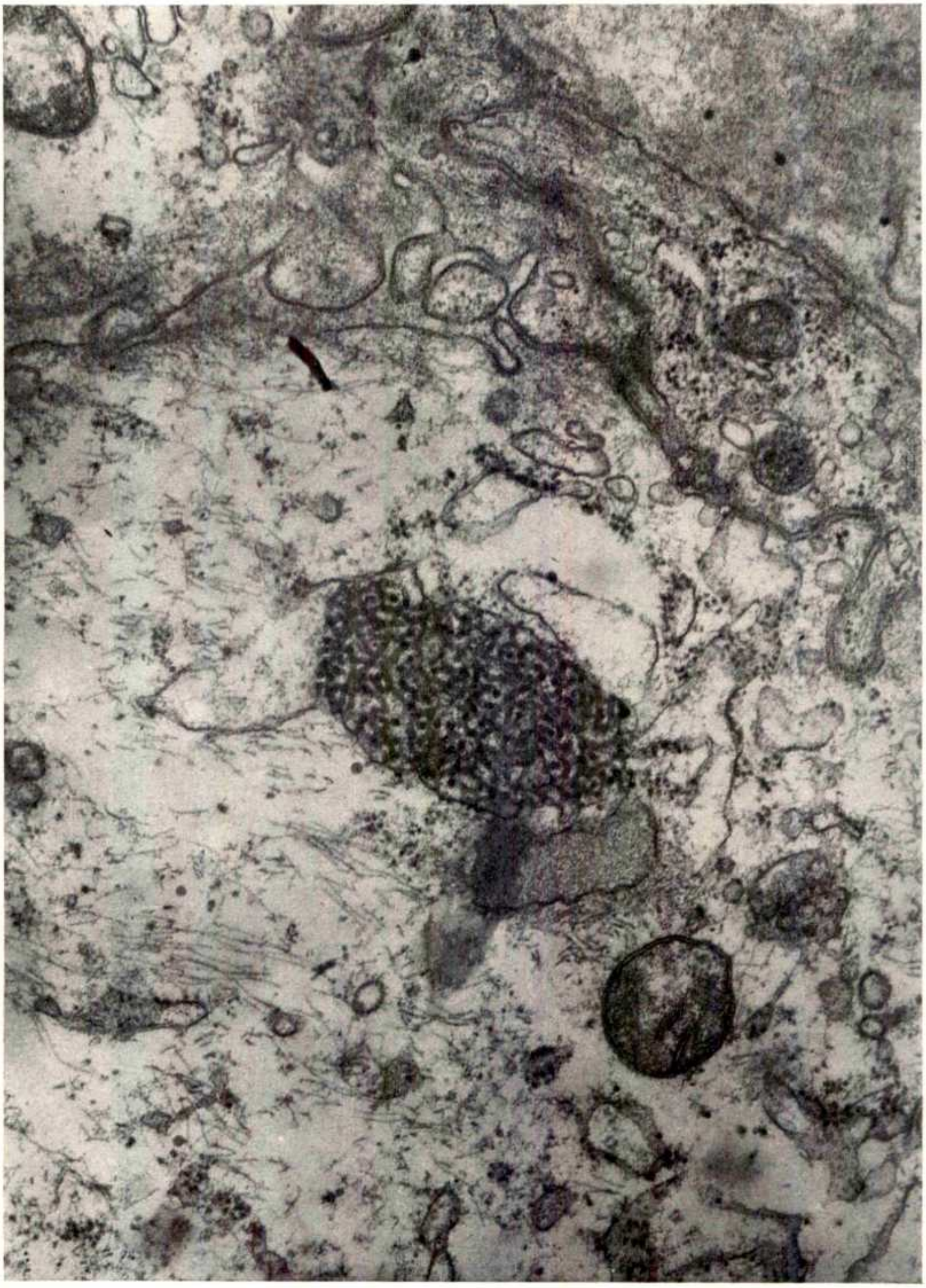

FIGURE 2. Electron micrograph of portion of endothelial cell showing the microtubular 'inclusion' within dilated rough endoplasmic reticulum. In addition, an adjacent segment of this organelle contains unorganized moderately electron-dense material (uranyl acetate and lead citrate, × 59,400).

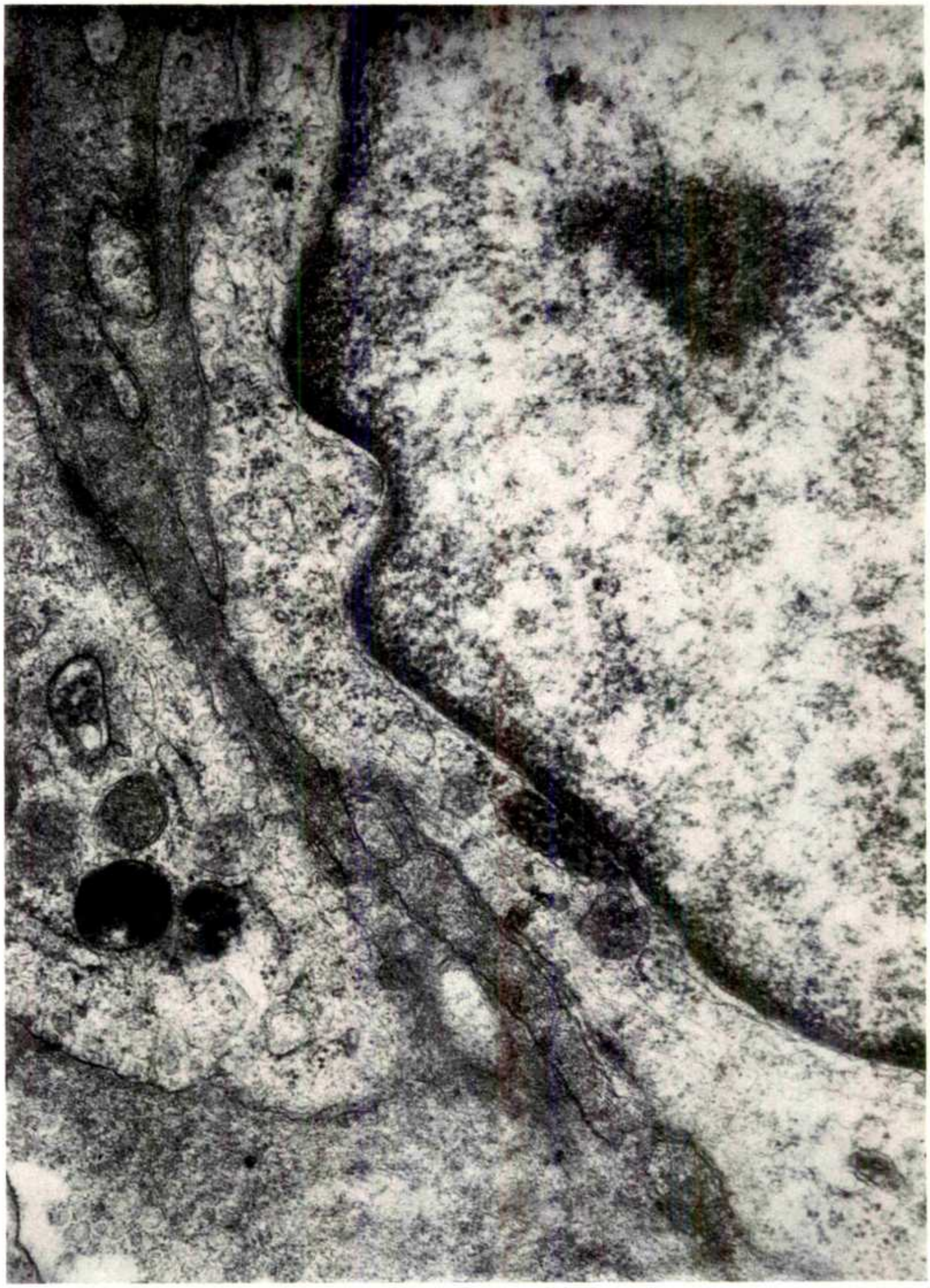

FIGURE 3. Electron micrograph of portion of endothelial cell showing microtubular 'inclusion' within the perinuclear space (uranyl acetate and lead citrate, × 48,600).

poliomyelitis, Aleutian disease, viral arteritis, hepatitis, leukaemia and Rous sarcoma. As seen in Table 2, the microtubular diameter ranges from 12 to 21 nm which is a smaller size range than that reported (20–25 nm) in all other previous related publications. This we attribute to variable shrinkage related to preparative techniques.

The available evidence indicates that the 'inclusion' is likely to be a product of the cell in which it is found. Alternatively the 'inclusion' could have been synthesized in a different (and even distant) cell, been liberated into the interstitial fluid, entered the circulation and been subsequently 'taken-up' by the cell in which it is seen at the time of biopsy. This latter hypothesis is unlikely as the 'inclusion' has regularly been located within the rough endoplasmic reticulum (RER) and peri-nuclear space. The RER, is the site of cellular synthesis of protein destined for subsequent 'export', and is never the location of materials which have been previously phagocytosed by the cell.

The original observation of 'inclusion'-positive endothelial cells in SLE has raised the question of the relevance of disordered vascular permeability. Could the 'inclusion' cause plasma leakage in collagenoses or might the 'inclusion' lodge in endothelium because of endothelial cell hyperpermeability? However, the endothelial cell is only one of many different cell types (*vide supra*) in which the 'inclusion' is found and although many of the associated diseases do have a vasculosis component, some do not. Search for the 'inclusion' in the commoner hyperpermeability states of diabetes mellitus and arterial hypertension might be rewarding.

The observations in the 'inclusion'-positive SLE cases have indicated (Haas & Yunis, 1970; Norton, 1969) that 'inclusions' are more frequent in active lesions than otherwise. This could be looked at in two ways; (1) causally-related 'inclusions' would be expected in highest numbers in active lesions; (2) vascular permeability would be maximal in active lesions and in consequence any invisible (soluble) circulating 'inclusions' could readily enter endothelial cytoplasm. In short the inclusion would be an electron microscopically visible indicator of hyperpermeability, in the same way that fibrin is a light microscopy marker of plasma seepage. The fact that the 'inclusion' material is found in non-endothelial cells (*vide supra*) is no barrier to a vasculosis-based argument. Fibrin which leaks from vessels in the vasculosis of malignant hypertension (Huttner, Rona & More, 1970) has been positively identified by electron microscopy within heart muscle cells adjacent to the abnormal blood vessels.

Since the 'inclusion' is a structure within a pre-existing morphological unit (the endoplasmic reticulum) it is natural to consider it as an 'intruder' rather than a normal cell organelle. Therefore, the presence of the 'inclusion' indicates abnormality. The 'inclusion' may, therefore, arise in three ways; (1) by active invasion of the cell (virus theory), (2) by passive trapping of circulating soluble 'inclusion' material in hyperpermeable vessels and (3) as a consequence of disordered function of the RER. It seems to us that the third possibility is the most likely.

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Post-inflammatory elastolysis and cutis laxa

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SUMMARY

Post-inflammatory elastolysis and cutis laxa (Marshall, Heyl & Weber, 1966) is a skin disease in African infants which appears to be comparatively common in at least two countries. Destruction of elastic tissue and atrophy are preceded by urticarial or by annular erythematous-papular lesions and result in severe disfigurement.

The clinical features are intermediate between anetoderma (macular atrophy) and acquired cutis laxa, but sufficiently typical and characteristic to constitute a distinctive syndrome, which might represent an abnormal reaction to the bite of an arthropod.

In 1966 Marshall, Heyl & Weber from South Africa described a disease in young children with features resembling anetoderma and cutis laxa, which they called 'post-inflammatory elastolysis and cutis laxa' (PECL). We wish to describe seven cases from Kenya.

CASE REPORTS

The seven girls came from the central highlands of Kenya; five from rural homes and two from urban districts. No history of a similar disease was recorded in their relatives, and the children had been healthy prior to the age of $1\frac{1}{2}$–4 years.

PECL is characterized by an acute inflammatory lesion followed by persistent elastolysis. The acute attacks lasted a few days or weeks only, occurring at intervals of up to 6 months and affecting different parts of the skin. In the mildest case (Case 1) the girl has not had further lesions during the past 2 years, after being affected for 9 months. The other patients have had recurrent attacks over a period of 2–8 years throughout the period of observation. The oldest girl is now 11 years old.

The morphology of the acute lesions was variable. Two main types could be distinguished. Firstly, lesions as described by Marshall *et al.* (1966) were seen in Cases 1, 2 and 3. An initial red papule spread to become an erythematous firm raised papular ring, 3–10 cm in diameter; the active margin was about 3–10 mm wide. At the centre the erythema and swelling regressed to be followed by hyperpigmentation and scaling which tended to fade leaving a fine white collarette (Fig. 1). The clinical course was capricious with attacks affecting different sites. Usually the activity and spreading of a lesion subsided quite suddenly, and the condition gradually changed to the chronic stage of the disease. This type of CEPL resembles erythema chronicum migrans.

In two patients (4 and 5) the lesions were more widely distributed and less well defined, consisting of erythema and swelling in geographical patterns. These plaques resembled urticaria, but they were firmer and lasted for several weeks. Such lesions did not show peripheral extension (Fig. 2). Case 6 had both types of lesion and details of the acute stage in case 7 were not available. Itching was not a prominent feature. Three patients (1, 4 and 6) had lesions resembling papular urticaria. Two younger sisters of Case 4 had typical severe papular urticaria simultaneously with the acute attacks of PECL in the proband.

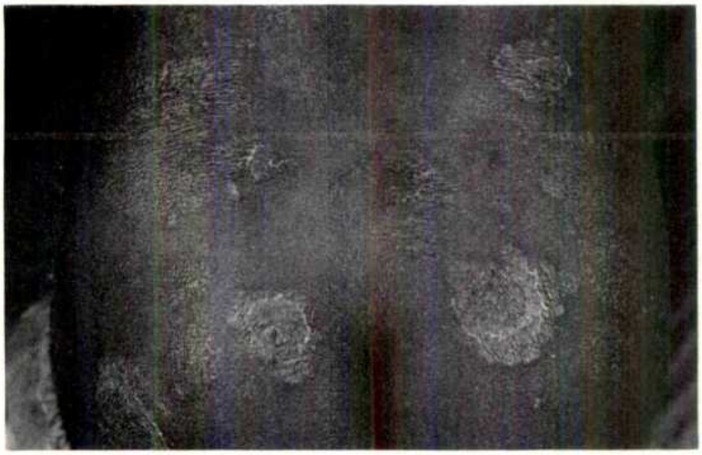

FIGURE 1. Case 2; several erythematous papular rings with a white collarette of scale; an older lesion (top left) already shows fine wrinkling and patulous follicles.

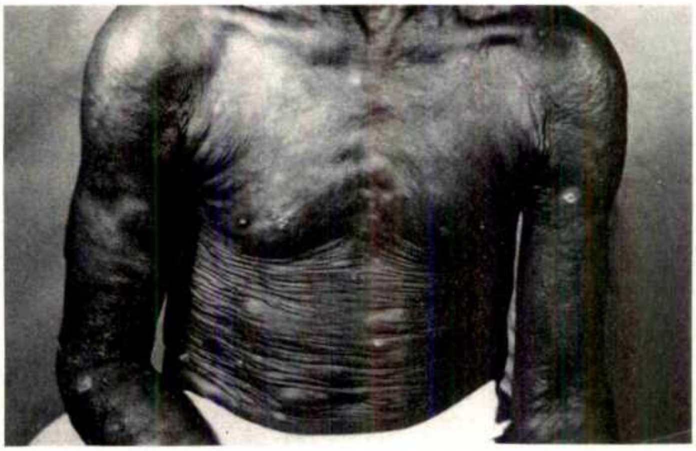

FIGURE 2. Case 4; widespread atrophy and elastolysis result from previous attacks, but there are also erythematous infiltrations and papular lesions with whitish scabs.

Admission to hospital resulted in rapid healing of the lesions and no further attacks were seen during a prolonged period in hospital.

The chronic phase which followed this apparently destructive inflammatory process was clinically

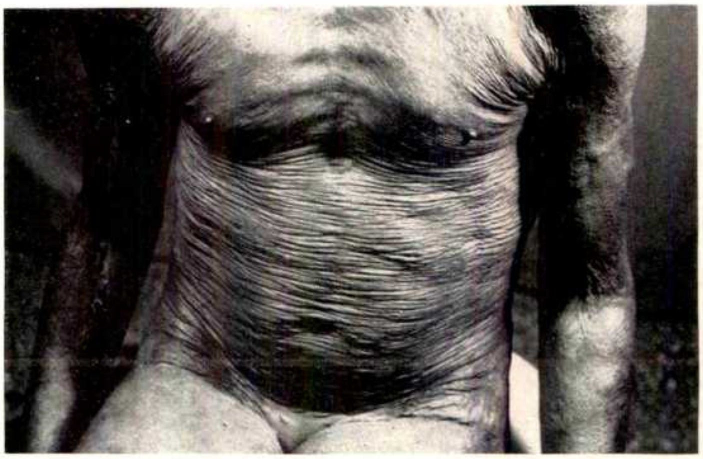

FIGURE 3. Case 4; quiet chronic stage; there is almost generalized wrinkling but no large redundant folds.

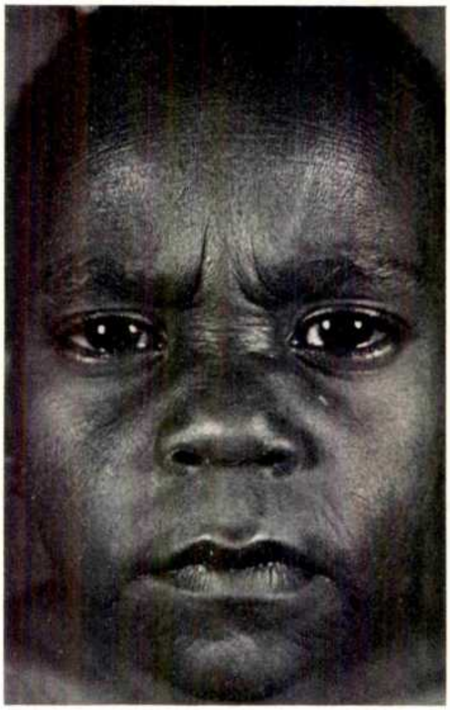

FIGURE 4. Case 3; the aged facial expression in one of the comparatively mild cases, a 4-year-old girl.

and histologically uniform but variable in severity. In the milder cases (Case 1) the lesions were localized with well defined gyrate and circinate margins; they often had a *peau d'orange* appearance. Wrinkling and loss of elasticity were obvious to the touch. In the most severely affected patient (Case 4) the loss of elasticity was almost universal and deep wrinkles gave the skin an almost senile appearance, resembling cutis laxa, particularly in the flexures of the neck and axilla (Fig. 3). When the skin was folded it remained in the same position for several minutes. Apart from loss of elasticity there was also atrophy, but this was not always conspicuous. There was no obvious depression of the skin, and the feeling of 'herniation' was hardly experienced in PECL. The affected skin was vulnerable; a small wound such as caused by a biopsy tended to gape and be surrounded by oedema.

The aged expression (Fig. 4) was a striking feature in all the children. Elastolysis was also prominent over the extensor aspects of the limbs and in some cases the distribution suggested sunlight as a cause. Lesions on the trunk developed more gradually or later, and were localized mainly on the shoulders and around the neck. The margins of the lesions were sharper on the trunk than on the distal part of the arms and legs; on the face the elastolysis lacked any well-defined demarcation. The scalp, palms and soles remained largely, but not entirely, free.

No other significant abnormalities were found. During the acute stage there was general malaise and slight to moderate fever, but without any consistent abnormalities of internal organs. The white cell count and ESR were raised with up to 10–20% eosinophilia. The lungs and the cardiovascular system were fully investigated in five of the patients to exclude internal complications as found in cutis laxa (chest X-ray, ECG, lung function tests, etc.), but no abnormalities were found. No hyperflexibility of the joints, skeletal changes or herniae were found. The intelligence of the children was normal.

The serological reactions for syphilis (VDRL and Kahn, and in 2 cases FTA-ABS) were negative. In some of the children various intestinal parasites or a positive Mantoux test were found, as expected in an African population. Although the distribution of the lesions suggested the influence of sunlight in some cases, provocation tests with high doses of ultraviolet light (Osram Xenon XBF 6000 lamp, distance 70 cm for 18 min, with Schottfilter WG6 of 2 mm) were negative. Screening tests for porphyrins and porphobilinogen (Eales, Levey & Sweeney, 1966) and analysis of the urine by means of ion-exchange chromatography (Doss, 1971; Doss & Schmidt, 1971) gave normal values at all stages. The parents and twenty-two siblings were investigated but no abnormalities were found.

Histopathology

Acute phase. In most sections the epidermis appeared normal except for slight spongiosis and infiltration with inflammatory cells. There was oedema of the upper and, to a lesser extent, of the mid-dermis accompanied by an inflammatory cell infiltrate arranged both diffusely and around the blood vessels (Fig. 5). The infiltrate consisted of polymorphonuclear leukocytes, lymphocytes, histiocytes and at times a considerable proportion of eosinophils. In places nuclear debris and necrotic foci were noted. In the upper dermis the capillaries tended to be swollen. The elastic fibres (Verhoeff and van Gieson Stains) were reduced in the upper dermis and some swelling and granular degeneration could be seen. Changes in the collagen were less apparent but some fragmentation and degeneration of the fibres were observed.

Chronic phase. The epidermis was normal or slightly atrophic. The dermis however was markedly reduced in thickness. There was a slight to moderate, mainly perivascular infiltrate of lymphocytes, with a few histiocytes, melanophages and mast cells. This infiltrate diminished in older lesions. The capillaries usually appeared normal, apart from occasional endothelial swelling. The elastic fibres were almost completely absent in the upper and mid-dermis. The remaining elastic fibres were thinner than

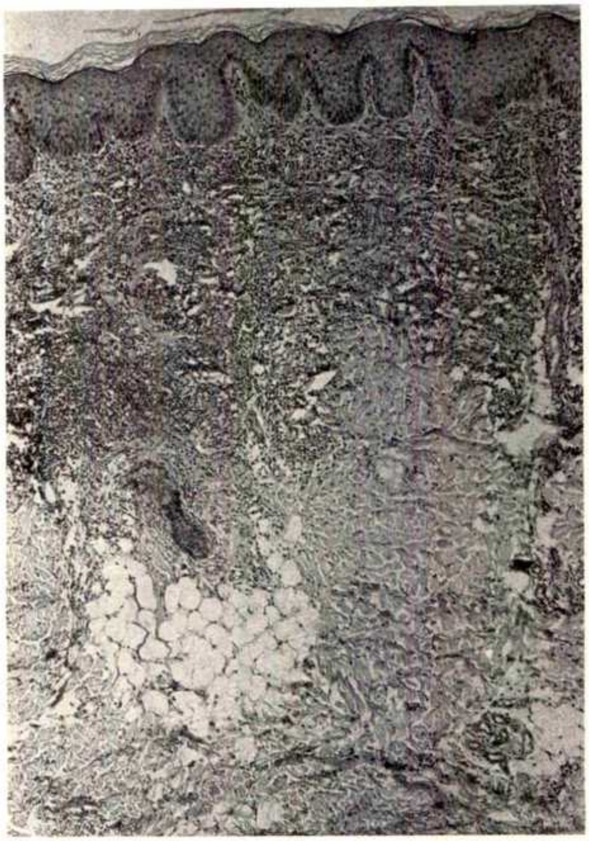

FIGURE 5. Acute phase; inflammatory infiltrate in the upper and middle third of the dermis (H & E, × 25).

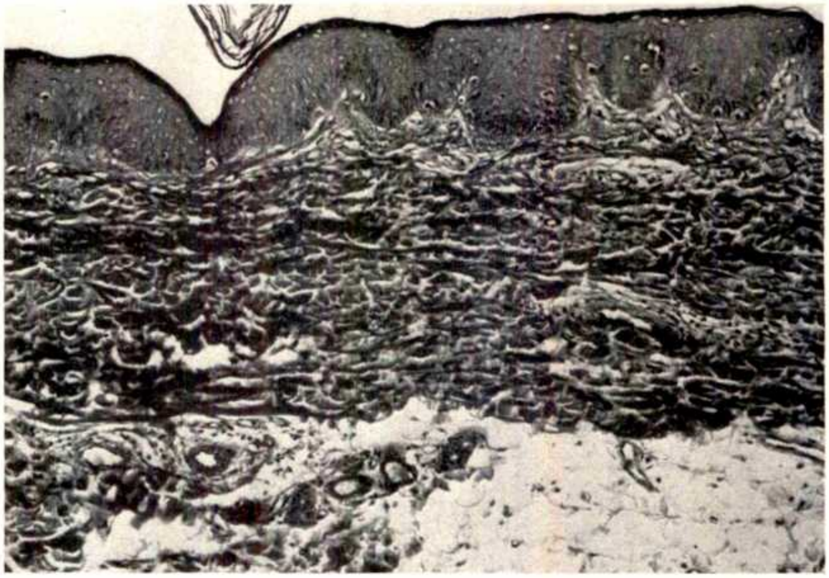

FIGURE 6. Chronic phase; the dermis is atrophic. The elastic fibres are swollen and fragmented with clumping (Verhoeff stain, × 50).

normal, and frequently fragmented and granular. Deep in the dermis they appeared swollen and fragmented with clumping (Verhoeff and van Gieson stains). The collagen fibres appeared atrophic and degenerate, especially in the upper two-thirds of the dermis. The epidermal appendages were normal, but were more superficial because of the dermal atrophy. In places the subcutaneous fat extended into the upper third of the dermis (Figs 6 and 7).

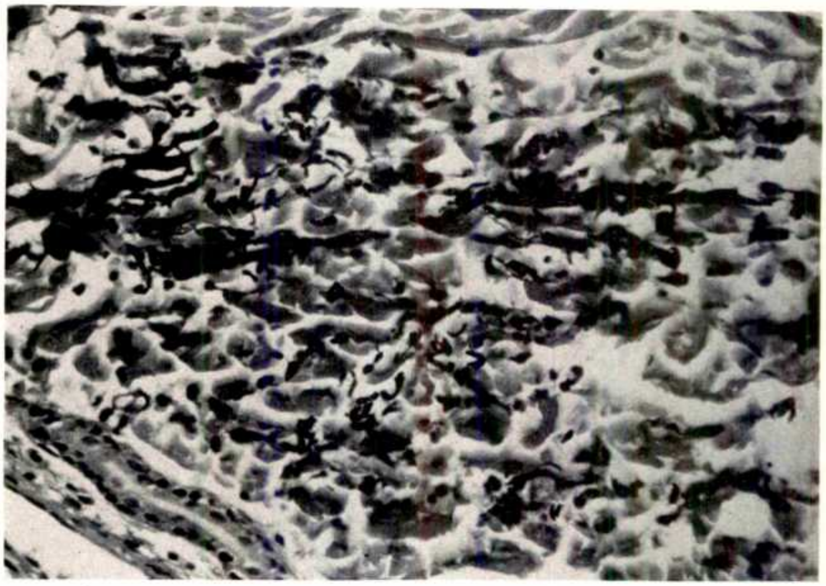

FIGURE 7. Chronic phase; Close-up of the degenerated elastic fibres (Verhoeff stain × 250).

DISCUSSION

Cases with similar symptoms and signs have been described under the diagnoses anetoderma or cutis laxa for more than a hundred years. These diseases with loss of elasticity and occasionally with atrophy of the skin are rare and also variable, which resulted in the introduction of new descriptive names for many of the single cases reported. Deluzenne (1956) attempted to simplify their classification.

Marshall *et al.* (1966) described five similar cases with 'stereotyped' features. Marshall & Gordon (personal communication, 1972) have since observed five other cases in Cape Town. They called the 'new' disease post-inflammatory elastolysis and cutis laxa. Ramos e Silva (1969) described an almost identical case in Rio de Janeiro. The description by Marshall *et al.* (1966) applies also almost exactly to our seven Kenyan cases. These eighteen cases can be characterized as follows:

(1) Almost all are girls (17/18). (2) All are Negroes or of mixed Negro-Asian-Caucasian descent (the so-called Cape-coloured of South Africa), living in tropical or subtropical climates. (3) The disease starts at the age of $1\frac{1}{2}$–4 years, in previously healthy infants with a negative family history. (4) The course is prolonged and is characterized by a relapsing acute inflammatory phase which lasts several months or years. (5) Morphologically, the acute phase consists of firm erythematous infiltrated lesions lasting about a week or fortnight, often as rings with a collarette of scales (most South African, three Kenyan cases) or as diffuse plaques (other Kenyan and the Brazilian cases); both types of lesion may occur together (Case 6). (6) In the affected areas this results in loss of elasticity, in laxity and usually in mild atrophy. A senile or aged facial expression is characteristic. In some cases most of the body surface

is involved. (7) Histologically there is an acute inflammatory infiltrate in the dermis and degenerative changes of the elastic and collagen fibres in the early phase; in the late phase the infiltration is less marked but there is atrophy of the dermis with marked degeneration of the elastic and collagen fibres. In the upper and middle dermis the elastic fibres are in most cases totally absent. (8) There are no consistent abnormalities of internal organs, or of the supportive connective tissues; involvement of the large arteries has however been described in one case with features of the chronic stage of PECL (Heyl, Simson & Cronje, 1971). (9) There is no evidence of any infectious disease to which anetoderma has been attributed, such as syphilis or tuberculosis.

Thus, PECL is a distinctive clinical syndrome peculiar in that it appears to be much more common in two African areas than are other types of acquired elastolysis described elsewhere. It may be added that we have seen several other similar children who were not well documented or investigated.

It is doubtful whether PECL represents a new nosological entity. Among the diseases with acquired elastolysis the most common is anetoderma or macular atrophy. Anetoderma has many features in common with PECL—a predilection for the female sex and an initial localization on the neck, shoulders and arms; a chronic relapsing course; an inflammatory stage characterized by urticaria (Pellizari type), red papular rings (Jadassohn type) or ill-defined erythema (many cases classified as Schweninger-Buzzi type); and no conspicuous abnormalities in internal organs. Like PECL it produces fine wrinkling, which has been compared to an empty raisin skin (Petges & Lecoulant, 1936); this is explained by the clumping, fragmentation and loss of elastic fibres in the upper dermis. The only striking difference is that anetoderma, as described in adults in temperate climates, is usually more localized and less severe than PECL. However, there may be numerous, disseminated lesions in anetoderma (Pellizari, 1884; Chargin & Silver, 1931; Petges & Lecoulant, 1936; Touraine, 1941; Joulia & Lecoulant, 1944; Deluzenne, 1956) and two of our cases were similar to generalized elastolysis as described in Europe (Souques & Charcot, 1891; Jablonska, 1966).

Genetically determined cutis laxa, in its dominant and recessive forms (Beighton, 1972), is a slowly progressive disease without an initial localized inflammatory phase; systemic involvement is usually found. We think that the clinical aspect is also different from PECL. The large loose folds have been described as 'pendulous' (Maxwell & Esterly, 1969) or 'redundant' (Grahame & Beighton, 1971); the facial expression has been compared to that of the bloodhound (Goltz *et al.*, 1965; Chadfield, 1969). PECL is characterized by fine wrinkling and a more truly senile appearance, although depending upon position and manipulation there may be folds (Figs. 2 and 3).

The cases recently reviewed by Reed, Horowitz & Beighton (1971) have been classified as 'acquired cutis laxa' and resemble the congenital disease closely. Acquired cutis laxa is mostly found in adults with an equal sex distribution; the initial inflammatory lesions are not circumscribed, the resulting large loose folds are of the bloodhound type and there is extensive involvement of internal organs.

In summary PECL shows features intermediate between anetoderma and acquired cutis laxa.

The aetiology of PECL is unknown, but it is possible that the disease is due to an abnormal hyperergic or allergic reaction to arthropod bites. In favour of this hypothesis are the course and the absence of new lesions when in hospital; the predilection for exposed parts; the eosinophilia in early skin lesions and in some acute cases in the blood; the simultaneous occurrence of papular urticaria in some patients and in other members of their families; the fact that erythema chronicum migrans and acrodermatitis chronica atrophicans often follow tick bites; and finally that PECL occurs at an age when African children start to play in grass and vegetation.

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Localized heat urticaria treated by inducing tolerance to heat

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SUMMARY

A patient with localised heat urticaria is described. Tolerance of the skin to heat was produced by repeated exposure to hot water and complete symptomatic remission was accomplished.

There are two ways in which urticaria may be produced by heat. First is the generalized form, or cholinogenic urticaria, which may follow exercise or generalized body heating and which may also be induced by emotional stress (Hopkins, Kesten & Hazel, 1938). Second is localized heat urticaria produced only by direct contact of the skin with heat and not by exercise or emotional stress (Duke, 1924). This second type is very rare (Jillson, 1956).

Systemic symptoms may occur if large areas of skin are affected at the same time in a person with localized heat urticaria. Nausea, faintness and loss of consciousness may develop with possible danger if such changes occur in a hot bath.

The physical urticarias as a group are amenable to treatment by repeated exposure of the skin to the causative agent so that a state of tolerance is induced. Such treatment has been successfully used in cold urticaria (Leigh, Ramsay & Calnan, 1974) and in solar urticaria (Obreshkova & Ramsay, unpublished observations). We report here the details of a patient with severe localized heat urticaria in whom we obtained complete symptomatic relief by producing tachyphylaxis to heat.

MATERIALS AND METHOD

The patient was a 36-year-old negress from Trinidad (L.W., P.31287), who had lived in England for 12 years. She gave a history that 6 months previously, 1 week after a hospital admission for tonsillectomy, she suddenly developed urticaria on exposure to hot water. This was severe and associated with nausea; it had recurred on every exposure to hot water, was localized to the areas immersed and caused swelling lasting 2–3 days. She had experienced two episodes of syncope whilst in a hot bath. She felt nauseated on exposure to sunlight but this had not produced any urticarial lesions. Drinking hot fluids caused numbness and irritation of the tongue. In the past she had undergone an appendicectomy, hernia repair and ovarian cystectomy 16 years previously, but her general health was now good apart from persistent pain in the jaw for which her tonsils had been removed. She was taking an oral contraceptive (Ovulen 50) and had used a sodium cromoglycate insufflator (Rynacrom) for rhinitis for several years but no other drugs had been taken. The rhinitis was persistent and unlike a seasonal hay fever. There was no other personal or family history of atopy. She was fit with no abnormality on general examination.

Investigations

Hb, 12·8 g/100 ml; W.B.C., 5,400/mm^3; no eosinophilia; E.S.R., 13 mm in 1 h; liver function tests, normal.

Tests with hot water. Immersion of the right arm in water at 40·5°C produced painful swelling limited to the immersed area, associated with nausea and faintness, after 5 min. The same arm was then immersed in water at 40·5°C for 1 min at hourly intervals and after five such immersions no urticaria developed.

Tests with monochromatic ultraviolet radiation and visible light (300–700 nm). No urticaria developed.

Tests with pharmacological agents:

Histamine. 10 μg injected intradermally into skin both before exposure to heat and after complete tolerance to heat had been induced gave identical normal weal and flare reactions.

Histamine liberator 48/80. An injection of 10 μg intradermally produced an identical normal weal and flare response both before and after the induction of tolerance to heat.

An area of skin not exposed to heat was then injected with 10 μg of 48/80 at 8 h intervals. After five such injections no response occurred. Exposure of the injected area to water at 40·5°C produced an urticarial weal after 1 min exposure. This test was repeated on a different area of skin with exactly the same result.

Poldine methosulphate. In an attempt to discover whether the reaction to heat was related to sweating an area of skin was treated with 5% poldine methosulphate in spirit and the area occluded with polythene overnight to suppress sweating (Grice & Bettley, 1966). The following day 10 μg of acetyl β-methyl choline was injected intradermally into normal skin and into the poldine treated site. Slight sweating occurred on the normal skin but not on that treated with poldine and no weal developed on the poldine treated skin. Exposure of the poldine treated area to water at 40·5°C for 1 min gave rise to a confluent urticarial weal.

Treatment

The arm in which tolerance to heat had been induced as described above was re-exposed to hot water after 2 h with no reaction, but immersion after a further 2 h produced urticaria. Treatment was recommenced 12 h later with immersions of the arm for 1 min every hour. After 2 h no reaction developed on that limb but the opposite untreated limb reacted to heat with an urticarial weal. The time between 1 min immersions was then gradually extended to 3, 6 and 12 h without urticaria appearing. The length of time of each immersion was then increased to 2 and 5 min and the time between immersions extended to 3, 6 and 12 h but no reaction occurred. The other limbs were then treated, one at a time, and then the lower and upper parts of the trunk. Each area was immersed for 1 min at hourly intervals until no reaction developed (usually 3–5 immersions). The length of each immersion was then slowly increased to 5 min at hourly intervals until urticaria failed to appear. The time between immersions was then increased to 3, 6 and 12 h. Once tolerance to heat of one part of the body had been induced it was maintained by immersion in hot water for 5 min every 12 h. When the whole body had been thus treated (a period of about 10 days) the patient was able to tolerate a bath at 40·5°C for 5 min without urticaria, nausea or syncope and the whole body immersion was repeated every 12 h. She then went to live in Malawi.

DISCUSSION

The usual advice given to patients with urticaria due to a physical agent is to avoid the cause and to take antihistamines systemically. It is possible, however, to treat some of these conditions by repeated exposure of the skin to the causative agent so as to induce a state of tolerance. Lehner & Rajka (1929) attempted to treat one patient with localized heat urticaria in this way using an infra red lamp with only limited success. In the discussion of the case of localized heat urticaria presented by Lewis (1939) it was suggested that tolerance to heat might be induced by exposing the skin to a slowly increasing temperature. Conversely, Moragas, Gimenez-Camarasa & Noguera (1973) did not observe a decrease in response to heat after three applications of heat to the same site in their patient. However it is possible that such a response might have been achieved if further exposures had been given.

There is no doubt that tolerance to heat was induced in our patient and this occurred within 10 days of starting treatment. The beneficial response that was achieved was not due to a spontaneous remission as was demonstrated by the fact that when one limb had been treated and was unresponsive to heat the opposite untreated limb reacted to heat with an urticarial weal.

Other physical urticarias have been treated by inducing tolerance of the skin to the responsible agent, e.g. solar urticaria (Obreshkova & Ramsay, unpublished observations) and cold urticaria, (Leigh *et al.*, 1974).

The basic mechanism underlying localized heat urticaria is unknown. Results of investigations in our patient suggest that it is not related to sweating. Similarly the mediator responsible for the vascular dilatation and increased capillary permeability is also unknown. It is of interest that when the skin was unresponsive to the histamine liberator 48/80, the urticarial response to heat in our patient was unchanged. In addition when the skin was unresponsive to heat it reacted in a normal way to 48/80. However, the action of 48/80 on the skin is complicated and it is not possible to draw any definite conclusions about the nature of the vasodilator material from these results.

Greaves *et al.* (1974) have recently reported a patient with localized heat urticaria and they produced evidence for histamine as the mediator. They also showed that the skin of their patient was refractory to heat after it had urticated from heat but they do not seem to have used this as a method of treatment.

Localized heat urticaria, together with the other physical urticarias, produces unpleasant symptoms and severe systemic reactions may at times be life threatening. These conditions are amenable to therapy which can lead to complete symptomatic relief. Controlled exposure to the causative agent rather than avoiding it may offer the best form of treatment.

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Pharmacology and Treatment

An explanation for the 'placebo' effect of bland ointment bases*

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SUMMARY

Bland topical applications, containing no pharmacologically active ingredients, were found to have an antimitotic effect on the epidermis of the stripped dorsal skin of hairless mice. White soft paraffin, cetomacrogol cream A (B.P.), starch paste, and emulsifying ointment (B.P.) were applied for 24 h periods under occlusive dressings to the backs of mice. The antimitotic effect was present at the time of removal of the dressings and up to 72 h after their removal. White soft paraffin had the maximal antimitotic activity and epidermis of mice treated with it showed only 23% of the number of mitoses of control animals having no such treatment. Emulsifying ointment had the least activity and in some circumstances actually seemed stimulatory to mitotic activity.

Bland ointment bases which do not contain any pharmacologically active ingredients often seem to assist the resolution of psoriatic lesions and patches of eczema. Indeed one of the difficulties encountered in assessing the effect of 'active topical preparations' is the so-called 'placebo' effect of the vehicles used in their formulation. In a previous investigation in which the antimitotic effect of topical corticosteroids was examined it had been found that a cream base used as a control topical application had a marginally significant antimitotic effect (Marks, Pongeshirun & Saylan, 1973). This paper is concerned with this antimitotic effect and confirms and extends these observations.

MATERIALS AND METHODS

Animal Model. Hairless mice of the Harwell strain (hr/hr) were used. Only male animals more than 4 weeks old were included. To induce a hyperproliferative epidermis (to serve as a model for psoriasis and chronic eczema) the dorsal skin was stripped of stratum corneum by repeated application of transparent adhesive tape until the 'glistening layer' was reached (Hennings & Elgjo, 1970). A measure of mitotic activity was obtained by injecting 0·15 mg colcemid (in solution) intraperitoneally 4 h before sacrifice. The colcemid stopped epidermal mitoses in metaphase so that the number of cells entering mitosis in a 4 h period could be determined. The number of cells in all phases of mitosis was expressed as a percentage of the number of basal cells (mitotic index). Care was taken not to count degenerating cells or basal clear cells which may appear superficially like cells in mitosis. At least 1500 basal cells were counted in each preparation. Several observers checked many of the histological sections and a good level of agreement was obtained.

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In several experiments an investigation was made of the effect of topical applications on the premitotic DNA synthesis stage of the generative cycle. Tritiated thymidine (Radiochemicals Centre, Amersham; Specific Activity 23 Ci/m mole, Batch 28 1 μCi/g mouse) was injected at the same time as the colcemid. Epidermal cells in the DNA synthesis stage incorporated the tritiated thymidine and were detected autoradiographically. Autoradiographs were prepared using the method of Joftes & Warren (1955). Ilford nuclear emulsion (K2) was used with exposure times of 3–4 weeks. The number of autoradiographically labelled basal and suprabasal cells was expressed as a percentage of the number of basal cells—at least 800 basal cells were counted—(labelling index).

Bland bases tested. The following applications were used:

(1) Cetomacrogol cream A (B.P.). (2) Emulsifying ointment (B.P.). (3) White soft paraffin. (4) Starch paste (25% Starch, 25% Talc, 50% W.S.P.).

Approximately 100 mg amounts of these preparations were placed on the backs of 'stripped' and 'unstripped' mice and kept in place during the period of application using an occlusive adhesive tape bandage. The applications were kept in place for 24 h in all these experiments. Observations were made at 24 h in most cases but in some instances the mice were sacrificed at 48 and 72 h after removal of the topical applications.

Epidermal thickness. The thickness of the epidermis was measured in a number of sections by determining the numbers of cells between and including the basal cell layer and granular cell layer at twenty random points (mean epidermal thickness).

RESULTS

All preparations tested, apart from emulsifying ointment, had an antimitotic effect after application for 24 h (see Table 1). The antimitotic effect persisted for at least 72 h after removal of the application on 'stripped animals' compared to the controls (see Table 2). The effect was also easily demonstrable autoradiographically (see Table 3).

Separate controls were necessary in this part of the investigation because of the wide range of labelling indices found in controls in experiments conducted at different times.

TABLE 1. The effect of various topical applications on the mean mitotic index of the epidermis of hairless mice after 24 h of application

Application	Condition	Mean mitotic index	Standard error of mean	Significance ($P=$) compared to control
None	Stripped	10·9	0·9	
Cetomacrogol A	Stripped	7·2	1·4	$0{\cdot}2 > P > 0{\cdot}1$
White soft paraffin	Stripped	3·7	0·6	$0{\cdot}005 > P > 0{\cdot}001$
Emulsifying ointment	Stripped	5·3	1·2	$0{\cdot}005 > P > 0{\cdot}601$
Starch Paste	Stripped	10·9	1·8	
None	Unstripped	2·6	0·6	
White soft paraffin	Unstripped	1·8	0·5	

The emulsifying ointment differed from the other preparations in not having an antimitotic effect. It actually significantly increased the labelling index.

The epidermal thickness resulting from each of the different topical applications is given in Table 4. There seemed to be a slight increase in epidermal thickness with all of the topical applications tested, save starch paste.

TABLE 2. The effect of white soft paraffin on the mean mitotic index of the epidermis of hairless mice 48 and 72 h after the removal of the application

Time after removal of application (h)	Application	Mean mitotic index	Standard error of mean	Significance ($P=$) compared to control
48	Nil	14·2	1·4	$0\cdot005 > P > 0\cdot002$
	White soft paraffin	9·3	1·6	
72	Nil	12·2	1·2	$0\cdot001 > P$
	White soft paraffin	5·6	0·9	

TABLE 3. The effect of various applications on the mean 'labelling index' of the epidermis of hairless mice after 24 h of application

Application	Labelling index	Standard error of mean	Significance ($P=$) compared to control
Nil	6·6	0·7	$0\cdot5 > P > 0\cdot4$
Cetomacrogol A	5·8	1·3	
Nil	8·1	0·5	$0\cdot01 > P > 0\cdot001$
White soft paraffin	2·4	0·5	
Nil	18·9	0·7	$0\cdot001 > P$
Starch paste	14·0	0·7	
Nil	11·5	1·9	$0\cdot1 > P > 0\cdot05$
Emulsifying ointment	15·5	1·2	

TABLE 4. The effect of various applications on the mean cell thickness of the epidermis of hairless mice after 24 h of application

Application	Mean cell thickness	Standard error of mean
Nil	3·8	0·2
White soft paraffin	4·5	0·2
Cetomacrogol A	4·1	0·2
Starch paste	3·1	0·1
Emulsifying ointment	4·9	0·2

DISCUSSION

It seems certain that physical occlusion in some way decreases an already enhanced mitotic activity in the epidermis. Apart from the results that we report above, Fisher & Maibach (1972) showed that adhesive tape decreased the mitotic activity of stripped human skin. In addition, Fry, Almeyda & McMinn (1970) and Baxter & Stoughton (1970) showed that occlusion with plastic film reduced the epidermal hyperproliferation found in psoriatic lesions. It is of interest that white soft paraffin was the most effective antimitotic application. It is of course the most greasy and 'stickiest' of all the preparations tested. This also accords with clinical experience that the most effective preparations in the treatment of psoriasis and chronic eczema are thick and greasy. We were slightly surprised to find that emulsifying ointment was not inhibitory to mitotic activity and was even stimulatory at times (see Table 3). This is perhaps due to the irritant effect that this preparation has in some patients. It contains sodium lauryl sulphate (3%) and this is known to 'irritate' the epidermis and cause an increase in cell proliferation (Polano, 1968). The mechanism of the antimitotic effect is of considerable importance because of the possibility of using it in a purposeful way for new dermatological treatments. Fisher & Maibach (1970) believed that the occlusion stopped the water loss from psoriatic lesions and that this could explain the antimitotic effect. While this may be a partial explanation we do not think it is the entire one. It is doubtful if our topical applications differed much in their occlusive properties with regard to water vapour. They were all applied under occlusive dressings and anyway cetomacrogol cream A is an 'aqueous cream'. In addition we found a decreased mitotic activity in unstimulated epidermis from topical applications (actually so did Fisher & Maibach at 3 days though not at 4). If there was a relationship between desquamation and cell production, greasy topical applications might well decrease cell loss just by their physical stickiness and the consequence would be a decreased mitotic rate. Thus we believe that it is possible that the rate of desquamation not only reflects the rate of cell production but can itself independently influence the rate of proliferation. Orfanos (1971) has employed this concept to explain the high output of epidermal cells in psoriasis suggesting that the primary disorder is one of 'stickiness' of the horny layer.

Whatever the explanation for the antimitotic effect of bland applications, its existence must be taken into account in clinical trials of topical preparations containing 'active' substances. In addition it seems likely that manipulation of the physical environment along the lines indicated may be worth exploring to develop new types of treatment for diseases involving the epidermis. Finally, it may be that the antimitotic effect of physical occlusion should be carefully considered and taken into account in explanations of the homeostatic mechanisms for epidermal growth.

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Double-blind controlled crossover high-dose study of Azaribine in psoriasis*

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SUMMARY

Azaribine used in high doses of 200 mg/kg a day is an effective agent in inducing temporary remissions in patients with severe psoriasis but potentially serious neurotoxicity may occur. Therapy should be initiated at lower doses of 125 mg/kg a day and advanced carefully only if clinical response does not ensue at the lower level.

Chronic psoriasis is a common and difficult medical problem which afflicts 1–2% of the population with considerable physical, psychological, and financial suffering. Since the discovery of increased mitotic activity in psoriatic lesions, attempts have been made to utilize systemic antimetabolites such as methotrexate, oral hydroxyurea, and topical nitrogen mustard to control the disease (Mandy, Taylor & Halprin, 1971; Moschella & Greenwald, 1973; Van Scott & Ekel, 1963; Weinstein & Frost, 1971). Toxicity and potential carcinogenicity limit the use of these agents, however (Harris, 1971; Mandy *et al.*, 1971; Moschella & Greenwald, 1973).

In a search for a less toxic chemotherapeutic agent, we studied 6-azauridine triacetate (Azaribine), which has shown no evidence of hepatic or renal toxicity and is reported to have almost negligible immunosuppressive activity (Calabresi, 1973; Vogler & Olansky, 1970). Pharmacological aspects of Azaribine have previously been discussed (Calabresi & Turner, 1966; McDonald & Calabresi, 1971).

Previous double-blind and open studies have been conducted with doses ranging from 70 mg/kg of body weight a day to 270 mg/kg a day (Turner & Calabresi, 1964; Vogler & Olansky, 1970).

In concert with other institutions studying Azaribine at various doses over 6-week courses of therapy, we investigated the use of 200 mg/kg a day in three divided doses (Milstein, Cornell & Stoughton, 1973a, b). Our goal was to compare results of therapeutic efficacy and toxicities with those of other institutions.

METHODS AND MATERIALS

Patient selection

During January to June 1972, twenty-seven patients with extensive uncontrolled psoriasis unresponsive to topical therapy were informed of the nature, purpose, and risks of a double-blind crossover study with Azaribine and placebo in consecutive 6-week courses. No patient with significant anaemia, or an ailment which might be exacerbated by an anaemia, such as coronary insufficiency or cerebrovascular ischaemia, was accepted. Women of childbearing potential were required to have gynaecological consultation and an appropriate contraceptive regimen was prescribed.

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Initial assessment included a medical history and physical examination, X-ray of the chest, urinalysis, complete blood count, platelet count, blood sugar, blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), and alkaline phosphatase. Skin biopsies were all consistent with a diagnosis of psoriasis. Photographs were taken, a manual sketch of lesions was drawn, and a 'rules-of-nines' calculation was carefully recorded for both the extent and thickness of lesions.

Study plan

Identical-appearing placebo and Azaribine tablets (500 mg) were packed in coded bottles for each 6-weeks' course of treatment and randomized so that neither the physician nor the patient knew the treatment sequence. At the end of 6 weeks, regardless of clinical response, the correspondingly coded crossover ration was issued for another 6 weeks of therapy. Topical petrolatum was the only additional medication allowed and a prolonged, cold New England spring precluded extra sun exposure.

Follow-up studies consisted of weekly clinic visits when clinical response and haemoglobin and white blood cell counts were determined. At 6 weeks, photographs and rules-of-nines calculations were repeated. At 12 weeks, photographs, rules-of-nines, blood sugar, BUN, and SGOT were again repeated. Upon the completion of the study, the patients were invited to return for monthly follow-up visits and some elected either to continue or to restart Azaribine on an open-study basis. Any patient who was selected for the study but was unable to tolerate the full dose of 200 mg/kg a day for 6 weeks was considered a treatment failure and was entered, if he wished, in a separate open-study group at lower doses.

Results were evaluated as follows: (1) 80–100% clearing of lesions, excellent; (2) 60–80% clearing, good; (3) 40–60% clearing, fair; (4) 0–40% clearing, no change; and (5) progession of psoriasis, worse.

Case material

Of the twenty-seven patients, eighteen were men and nine were women. Two young male patients were casual in keeping weekly follow-up appointments and were dismissed from the study. A fifth episode of diverticulitis developed in one middle-aged woman while a placebo was being administered

TABLE 1. Prior antimetabolite therapy

Antimetabolite	Number
Methotrexate (12/24 patients)	
Good control	1
Partial control	2
No effect	2
Significant toxicity	6
Inadequate trial	1
Hydroxyurea (2/24 patients)	
No longer responsive to therapy upon retreatment	2

before she received any active drug; the patient was dismissed from the study because her code was broken in an attempt to establish any drug responsibility.

The remaining twenty-four patients were characterized by an average age of 48 years and averaged a 20-year duration of psoriasis. Twenty-one patients had generalized plaques, one had generalized arciform plaques, and two had erythroderma. Nine patients had psoriatic arthritis. Prior antimetabolite therapy is summarized in Table 1.

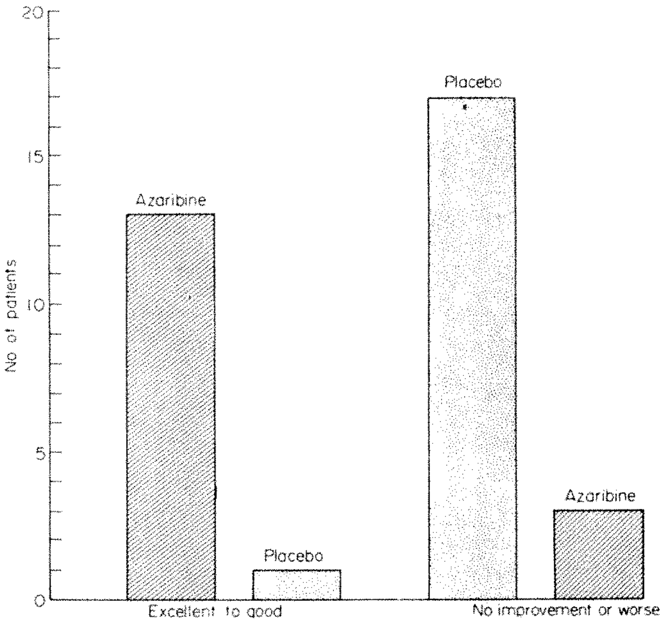

FIGURE 1. Six months' double-blind study of Azaribine.

RESULTS

Results are outlined in Table 2 and Fig. 1. Thirteen patients achieved good or excellent improvement while receiving Azaribine compared with one good response in patients receiving a placebo.

Patients given Azaribine usually showed noticeable improvement between 7 and 14 days of therapy and tended to reach a maximal rate of improvement between 3 and 5 weeks of therapy. Extrapolation of average improvement curves for the entire series reached 100% at approximately 8–9 weeks of treatment. Mean improvement after the six weeks of Azaribine was 82% clearing. Residual lesions at that time were usually in the form of macular erythema or minimally raised almost non-scaling plaques. Of the ten patients who received Azaribine in the first 6 weeks, five maintained improvement during the 6-week placebo period while another five relapsed after an average of 34% of that time. Follow-up of those who successfully completed the study showed that four out of eighteen relapsed in 1–2 weeks, seven out of eighteen remained clear for at least 1–3 months, and three out of eighteen remained clear until lost to follow-up or relapse from 3½ to 8 months after treatment. Four out of eighteen either restarted or continued treatment to obtain greater improvement.

Toxicity. Major toxicity is summarized in Tables 2 and 3. The most serious side effect at this high

TABLE 2. Cohort analysis of and summary of side effects from Azaribine double-blind control study at 200 mg/kg a day

	Treatment Phase	
Response	Azaribine	Placebo
Clinical		
Excellent	5	0
Good	8	1
Fair	2	0
No change	3	9
Worse	0	8
Total	18	18
Haematological		
Haemoglobin dropped to 11·0 gm (3 patients had pretreatment levels of 12·5, 12·6, and 12·7)	11	0
Leukopenia less than 4,000/mm^3	1	0
Thrombocytosis (transient)	1	0
CNS transient		
Fatigue (moderate to severe)	8	0
Insomnia	3	0
EEG abnormalities	2	0
Dysequilibrium	1	0
Slurred speech	1	0
Depression	1	0
Other transient side effects		
Antabuse effect	11	0
Moderate nausea	3	1
Arthritis flare	2	1
Aphthous ulcers	2	0
Decreased taste for tobacco	2	0
Herpes simplex	1	0
Herpes zoster	1	0
Decreased libido	1	0
Decreased beard growth	1	0
Flu syndrome	0	1
Chest pain 3 days	0	1

dose was the development of toxic encephalopathy which was characterized by severe lethargy, slowing of intellectual response to questioning, diplopia, dysequilibrium, and electroencephalographic changes. Despite warnings to stop taking the medication immediately and call the attending physician if confusion or severe fatigue ensued, most patients ignored these changes in order to experience further improvement. In all, six out of twenty-four patients showed electroencephalographic abnormalities and four of them required hospital observation. Two of these patients were clinically semicomatose. In each instance, the patient recovered in several days after cessation of therapy, and one later restarted the medication at a full 200 mg/kg a day without experiencing severe fatigue again. The central nervous system symptoms appeared gradually, but unpredictably, at any

TABLE 3. Major toxicity

		Haematopoitic				
Group	Dosage (mg/kg/day)	Anaemia	Leukopenia (below 4,000 mm^{-3})	Platelet disorders	Central nervous system	Gastrointestinal system
Milstein *et al.*	125	33–34% drop in haematocrit in 9/21	None	None	11/21	11/21
Vogler & Olansky	200	Not comparable	1/22	1/22 transient thrombocytopenia	3/22	10/22
Crutcher & Moschella (present study)	200	Less than 11 gm Hb; 5/18 macrocytic	1/18	1/18 transient thrombocytosis	13/24	7/24

time during the 6-week Azaribine phase of the study period. Six of the twenty-four patients experienced considerable nausea which resulted in a reduction of dose and were entered in a lower dose open-study group. An anaemia of less than 11·0 gm of haemoglobin developed in five patients during the Azaribine phase of the study. Three of these patients had a macrocytic normochromic anaemia, one had a normocytic normochromic anaemia, and in one patient, who had pre-existing thalassaemia, a relative macrocytosis occurred. Recoveries occurred spontaneously within several weeks of cessation of therapy. A leukopenia of 3,200 mm^{-3} developed in one patient during administration of Azaribine, which resolved spontaneously 1 week after finishing the Azaribine phase of treatment. A thrombocytosis of 880,000 mm^{-3} developed in another patient, but the count returned to normal levels when retested 6 weeks after discontinuing active therapy.

Two alcoholic patients were noted to have abnormal SGOT levels at cessation of therapy. In one, this level rose from 34 to 63 units concurrently with drinking while taking Azaribine. In the other, the level fell from 164 to 71 units, and the patient continued drinking without experiencing an antabuse effect while taking Azaribine. No patient demonstrated a rise in BUN. Two patients experienced a decreased taste for tobacco during the Azaribine phase of the study. Table 2 lists isolated complaints that might be related to the medication.

Treatment failures. Table 4 summarizes treatment failures. Central nervous system symptoms or a combination of central nervous system and gastrointestinal symptoms were responsible for five of these failures; three of these patients were hospitalized for observation of toxic encephalopathy. Another patient with diffuse arteriosclerosis experienced extreme nausea from the drug and was excluded from the closed study; however, the patient was later retreated in an open-study programme, and an axillary artery thrombosis developed after he strenuously exerted himself trying to start a power grass mower.

DISCUSSION

As seen in Table 2, Azaribine was a highly effective drug in inducing temporary remissions in patients with severe psoriasis. A comparison of results using 125 mg/kg a day versus 200 mg/kg a day showed no therapeutic advantage to the use of the higher dosage, however (Table 5, Milstein *et al.*, 1973a). On

TABLE 4. Treatment failures

Age (years)	Weeks on Azaribine	Limiting toxicity
63	1	EEG abnormalities, nausea
42	2	Fatigue and nausea
56	2	EEG abnormality, dysequilibrium, fatigue, nausea
54	4	EEG abnormalities, seizures
61	4	Nausea, axillary artery thrombosis
51	6	EEG abnormalities, epigastric burning

TABLE 5. Results

Group	Dosage (mg/kg/day)	Initial response observed (weeks)	Drug effectiveness	Duration of response
Milstein *et al.* (1973)	125	1	Greater than 60% clearing in 86%	Not stated
Vogler & Olansky (1970)	200	2	Greater than 75% clearing in 73%	Average, 5 1/2 weeks
Crutcher & Moschella (present study)	200	1–2	Greater than 60% clearing in 72%	Mean, 5 1/2 weeks

the other hand, toxicity to the central nervous system was of far greater severity in our study at the higher dose levels (Tables 2 and 4).

We believe that high doses of Azaribine should be reserved for those patients whose condition is unresponsive to the lower dose regimen and for those who have considerable physical, social, or psychological handicaps as a result of their disease. Close evaluation of patients with significant premonitory lethargy should be instituted and the dosage adjusted appropriately.

ACKNOWLEDGMENT

We wish to thank Howard Milstein for providing additional data concerning his study subjects.

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Case Reports

Juvenile melanoma disseminatum

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SUMMARY

A young man with juvenile melanoma disseminatum is described. The eruption appeared when he was 18 years old and was resolving 12 years later.

Juvenile melanoma usually is solitary (Spitz, 1948); however some authors have mentioned two or more lesions in the same patient (Table I).

CASE REPORT

The patient, aged 22 years, a mechanic from Crete, was first seen in 1965 with an eruption which had appeared 4 years previously when he was 18 years old. He had a non-itchy eruption mainly on the buttocks, thighs, and arms but some lesions were on the forearms, the legs, and the trunk (Fig. 1). There were many small, firm, discrete nodules ranging in colour from dark brown to that of normal skin. The surface of most lesions was smooth, in others it was slightly granular, while others had a verrucous appearance (Fig. 2). The nodules were asymptomatic.

Skin biopsies were done on various lesions. Histopathological examination showed changes similar to those of a cellular naevus or juvenile melanoma. Hyperkeratosis, acanthosis, elongation of the rete ridges and junctional activity were seen. The dermis was oedematous and infiltrated with naevus epithelioid cells and multinucleated giant cells (Figs 3 and 4).

Some of the lesions were destroyed by electrocoagulation, only to reappear after a few months. By December 1967 no further new nodules had developed and the others were slightly smaller. For 1 year, from November 1967, he had several epileptic fits every day. He was admitted to a neurosurgical department for investigation (Mr J.Taptas) but no organic brain lesion was detected.

X-rays of the skull, cervical and thoracic spine were normal. The EEG showed moderate bilateral abnormalities with a slight preponderance on the left. The brain radioisotopic scanning with ^{207}Hg was also normal.

Whether the epileptic fits were related to the multiple disseminate juvenile melanomata is not clear. The onset of epileptic fits for the first time without obvious cause at the age of 24 years suggests that they are related. (Dr. C. Argyropoulos.)

Further support for this relationship comes from a follow-up examination (April 1974) when the skin lesions were found to have considerably regressed in size without any treatment, and the patient was free of any epileptic attacks.

TABLE I.

Case reports	Sex	Age (years)	Number of lesions	Location	Remarks
Traub (1949)	M	10	Several small dark spots around the injection site	Right arm	After anti-whooping cough inoculation
Duperrat & Goetschel (1958)	M	3	20 hard nodules each about 5 mm in diameter	Right nostril	
Duperrat & Dufourmentel (1959)	F	3	One infiltrated circumscribed plaque, with several nodules on it.	Posterior surface of left arm	After sunbathing. 'Multiple forme agminée'
Doepfmer (1960)	F	10 months	2 lesions	Right cheek	
Winkelmann (1961)	M	7	4 lesions on a plaque	Left cheek	
Jablonska *et al.* (1965)	F	50	Multiple nodules developed into one plaque with an irregular surface	Nose	After excision of preexisting juvenile melanoma and plastic repair
Kopf & Andrade (1966)	F	2	3 lesions	Right wrist	
Bureau *et al.* (1964)	F	8	2 lesions at the age of 15 months. After excision, many dark grey spots appeared.	Right cheek	At the age of 7 years, 2 new similar lesions on the right eyelids
Korting *et al.* (1968)	F	5	25 multiple agminate lesions on hypopigmented plaque	Left side of the back	
Sourreil *et al.* (1969)	M	7	5 nodules	Left cheek	'Multiple forme agminée'
	M	10	5 nodules	Left side of the back	'Multiple forme agminée
Bourlond (1971)	M	6	Multiple lesions	Thighs, buttocks	Yellowish red maculo-papules
Brownstein (1972)	M	2½	Large number of tumours in one group	Left cheek	
Wallace (1974)	F	49	Multiple lesions	Widespread	

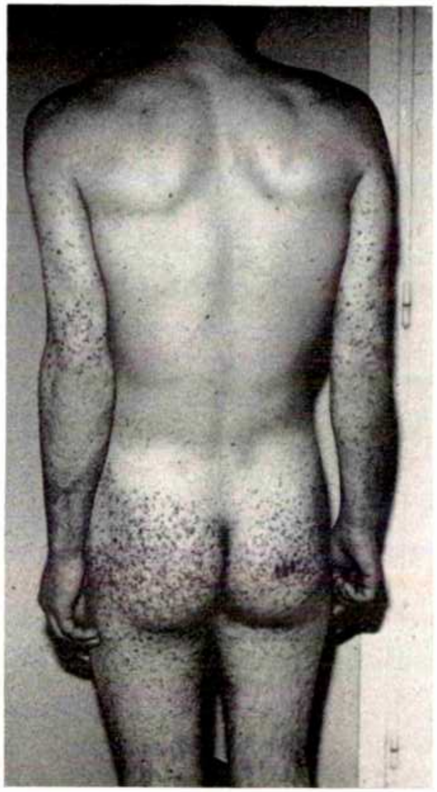

FIGURE 1. Lesions of juvenile melanoma disseminatum on the posterior aspects of the body.

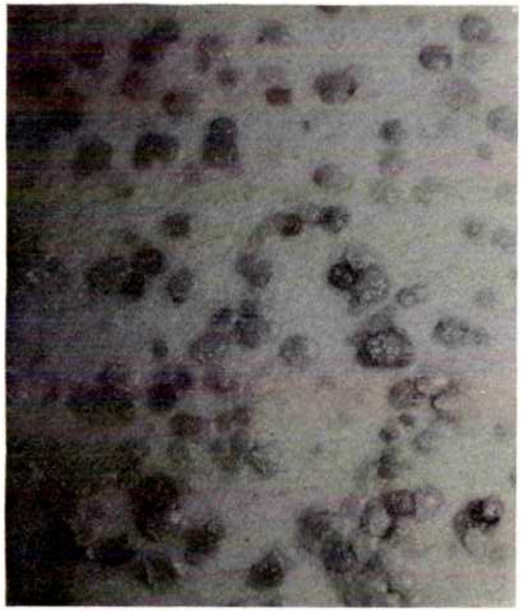

FIGURE 2. Close-up of lesions.

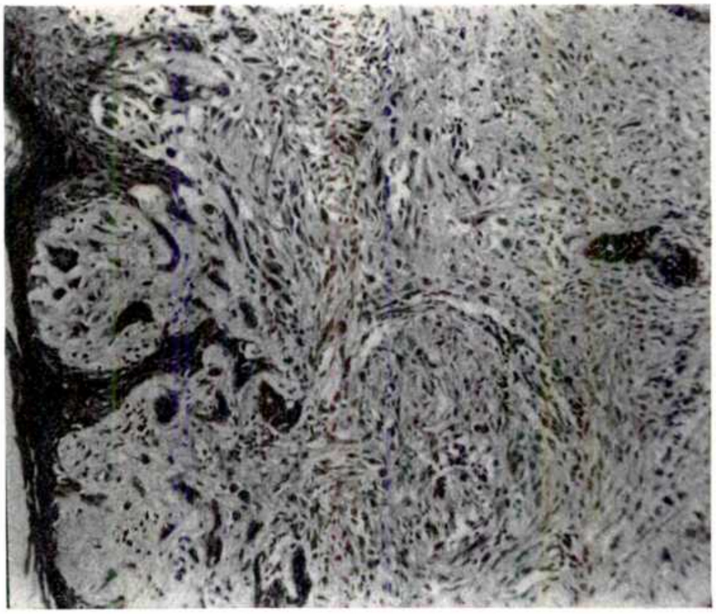

FIGURE 3. Histopathology of juvenile melanoma disseminatum. There is junctional activity and the corium is infiltrated by naevus epithelioid cells and multinucleated giant cells.

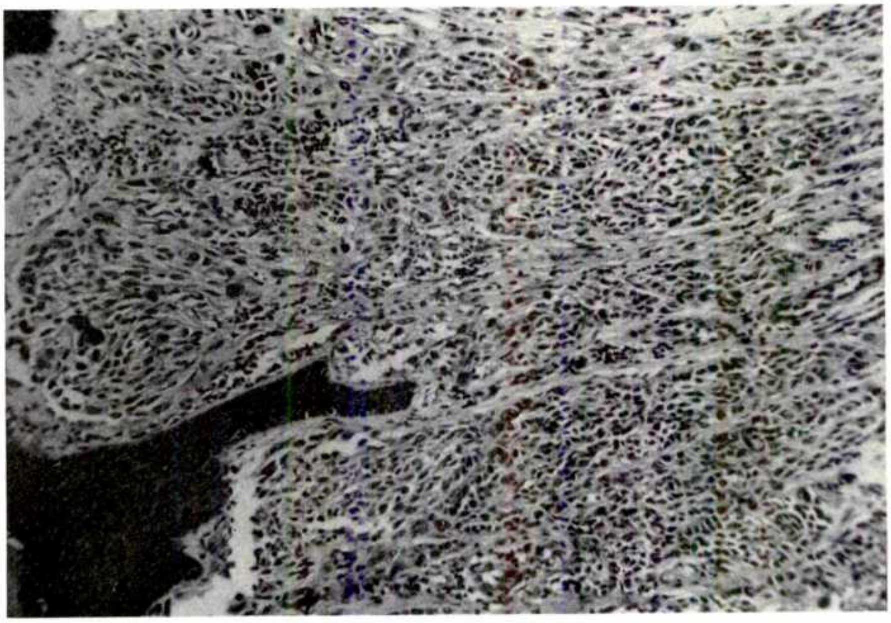

FIGURE 4. Histological features of another part of the lesion.

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A fixed drug eruption due to paracetamol

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SUMMARY

A fixed drug eruption due to paracetamol is reported.

Over 10,000,000 prescriptions (EC10) for paracetamol are issued annually and rashes occurring as possible adverse reactions to this drug are reported fairly frequently to the Committee on Safety of Medicines (Committee on Safety of Medicines—personal communication). These include urticaria, angioneurotic oedema, purpura, morbilliform and scarlatiniform rashes, erythema nodosum, eczema, alopecia and nail changes but in many of these cases the cause–effect relationship is unproven. One strongly suspected case (Savin, 1970) was reported from St John's Hospital. This was a patient who developed a fixed drug rash after taking a chlormezanone–paracetamol combination. However, no other reports of a fixed drug rash which was proved to be due to paracetamol have been found.

CASE REPORT

An Asian lady (Mrs A.K.) aged 34 years was first seen in August 1973. For the previous 18 months she had suffered from a patchy itching rash. This occurred in attacks which involved the same areas of skin on each occasion. Attacks originally lasted a few days and usually coincided with a menstrual period. In the course of time, however, they got gradually longer so that there were no remissions between them.

On examination she was found to have between forty and fifty bluish brown macules scattered over the trunk and limbs and particularly the lumbar area and hips. They were mostly round or oval and varied in size from 1–4 cm. The appearance was characteristic of a fixed drug eruption.

On questioning it was found that she took two tablets of paracetamol B.P. regularly every 2 or 3 weeks and no other medicines except for a few iron tablets.

When she stopped taking paracetamol the rash immediately improved, and cleared during the next month leaving only some residual pigmentation. A challenging dose of two 500 mg tablets was followed by itching within half an hour. This affected the same sites as before and lasted 3 days. After a 2 day interval she took 2 more tablets and the itching recurred followed by a macular rash identical to the original one.

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She has taken no more paracetamol and there has been no recurrence of the rash.

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The smell of cancer

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SUMMARY

A middle aged man presented reluctantly to his General Practitioner with the complaint of offensive body odour and he was referred for a dermatological opinion. On examination a malignant looking ulcer emitting a foul odour was found in the site of his penis.

CASE REPORT

A 55-year-old man was persuaded in 1973, following insistence by his colleagues at the Brewery at which he worked, to see his General Practitioner because of the unpleasant smell he emitted. His body odour quickly permeated the surgery, yet he claimed he bathed nightly; he was referred to the Dermatology Department on the supposition of excessive sweating and the possibility of him being a candidate for sympathectomy.

At hospital his offensive odour was readily apparent. Reluctantly a huge ulcerating lesion in the suprapubic region was revealed and his penis had been totally destroyed. He had first noticed a small ulcer on the glans penis in 1971; he did nothing about it and the lesion had gradually enlarged and eventually eroded the entire penis and surrounding skin. His urine flowed from the centre of the resulting necrotic crater, but he denied dysuria; although he suffered urgency of micturition he avoided incontinence. His general health was good and in the past he had not been circumcised nor had a urethral discharge.

Examination suggested the foul odour was not merely coming from the necrotic cavity but from him as a whole. The odour persisted despite constant change of personal and bed linen.

He was clinically anaemic and had bilateral ankle oedema but there was no lymphadenopathy or hepatomegaly. There was a large ulcerating, apparently malignant cavity, with a pool of pus, which had destroyed the penis. It extended from right to left inguinal regions and reached from the upper scrotum to above the level of the symphysis pubis. There was a 3 cm indurated margin all round the ulcer edge. The scrotum was enlarged, oedematous and indurated (see Fig. 1). Examination under anaesthetic showed that the crater extended almost to the anal margin and the urethra was not identifiable. Rectal examination revealed that the tumour was fixed to the pubo-ischial ramus on the left. Biopsy was taken of the ulcer margin.

Investigations

Hb 8·2 g/100 ml; WBC 12, 100 mm^{-3}; ESR 50 mm/l h. Electrolytes and urea normal except Na 132

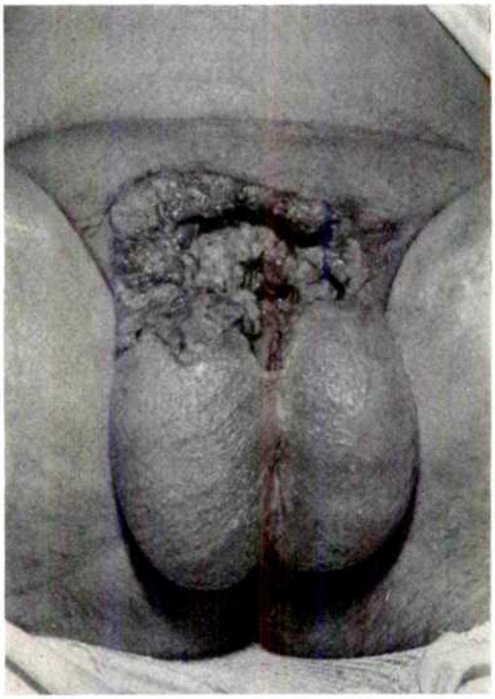

FIGURE 1. Initial appearance before surgery.

mEq; alkaline phosphatase 16 K.A. units/100 ml; WR, VDRL, RPCFT were all negative and the results were checked. X-ray of pelvis showed poor definition of the left inferior pubic ramus—suggesting early bone involvement by tumour or infection. A swab from the lesion grew β Haemolytic Streptococci. Histology of the biopsy showed poorly differentiated carcinoma.

MANAGEMENT AND PROGRESS

After blood transfusion a supra-pubic cystostomy was performed and the scrotum and testes were removed—leaving a necrotic cavity. Pathological examination of the excised tissue showed a fungating tumour in the upper scrotum with tumour on the spermatic cord. Histology revealed this to be a squamous cell carcinoma and there was testicular fibrosis resulting from ischaemia.

He was given a course of radiotherapy which was discontinued eventually due to severe diarrhoea. When commenced on intramuscular Bleomycin he developed a severe pyrexia with disorientation, therefore the treatment was stopped. By this time his body odour was certainly not quite so offensive. A fluctuant, non pulsatile swelling developed in the right groin, which eventually drained into the cavity and was treated with antibiotics. His general condition gradually deteriorated and he died in April 1974.

DISCUSSION

This man's symptoms began 2½ years ago, but only when his colleagues refused to work with him because of his odour did he reluctantly see his General Practitioner; he evaded the real issue and misled his doctor to think in terms of excess perspiration. He had kept his condition a secret from his wife;

he had been married only $3\frac{1}{2}$ years and had no children. Blunt questioning eventually revealed the reasons for this man's uncommunicative attitudes. During World War II in Italy he had developed white lesions on the glans penis; he sought medical advice and was told he probably had venereal disease and was given injections and tablets and the condition cleared. The inference is that he feared that the venereal disease had recurred in 1971, only a year after his marriage. Neglect of his lesion when it was at a curable stage through fear and shame had lead to gross mutilation of his anatomy and an early death. It is indeed ironic that all his tests for venereal disease were negative.

To our personal knowledge, other instances of odour being a presenting sign of malignancy have been neoplasms of the female breast and of the ear; both sites being supplied with modified apocrine glands. There is the case of the Nursing Sister who was noticed to have an unpleasant body odour by the nursing staff and eventually it transpired that she had a fungating carcinoma of the breast which she kept concealed for some time by packing it with cotton wool swabs. Another parallel case was an elderly lady in an Old Folk's Home who was noticed to have an unusual smell and when her hair was lifted she was found to have a large basal cell carcinoma on her ear.

Bromhidrosis due to a nasal foreign body has been reported (Golding, 1965). This case report is not unique as two other children presenting with malodourous eccrine sweat were each found to have a foreign body in their nose (Champion, 1974—personal communication).

Body odour in the main is produced by apocrine glands' secretion, particularly those in the axilla because those in the pubic area are physiologically inert even when they are anatomically perfect (Kligman & Shehadek, 1964). Unless one is a garlic eater eccrine secretion is usually without smell but hyperhidrosis whatever the aetiology can produce an offensive odour. It is possible in the case described in this paper, that some substance was being absorbed from the necrotic lesion and excreted in the eccrine sweat to produce the offensive body odour.

ACKNOWLEDGMENT

We would like to thank Dr H.MacDonald, Consultant Radiotherapist, Southampton.

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Tylosis following post-corrosive stricture of the oesophagus

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SUMMARY

A case of tylosis following corrosive stricture of the oesophagus in a male of 26 years is recorded. It is suggested that there is probably a connection between the state of the oesophagus and the state of palms and soles and that an oesophageal abnormality may precede tylosis of the late onset type.

Howel-Evans *et al.* (1958) reported the association of tylosis (hyperkeratosis palmaris et plantaris) with carcinoma of the oesophagus. In their study of forty-eight members of two families in Liverpool, who had developed tylosis of the late onset type, seventeen developed a squamous cell carcinoma of the oesophagus. Clarke *et al.* (1959) in a review of these cases estimated that the healthy tylotics of these two families had a 95% chance of developing carcinoma between the ages of 26 and 63 years. Shine & Allison (1966) reported a carcinoma of the oesophagus occuring in a tylotic in his 61st year of life. He had a congenital abnormality of the oesophagus with a sliding hiatus hernia and gastric mucosa at the lower end of the oesophagus, wherein he developed a peptic stricture. This stricture later on became the site of a carcinoma. He had developed dysphagia at the age of 18 months, followed by tylosis in adolescence.

In the case reported here, tylosis developed in a patient after a surgical by-pass carried out for a post-corrosive stricture of the oesophagus with dysphagia.

CASE REPORT

M.V., male, aged 26 years was seen because of thickening of the palms and soles and ichthyosis of the trunk and limbs, both of about 3 years' duration. On enquiry it was elicited that 6 years before, when he was aged 20 years, he attempted suicide by swallowing sulphuric acid which gave him some acid burns on his face. A few months later he developed a post-corrosive stricture of the oesophagus. This disability was treated with a gastrostomy, but a few months later he was subjected to major reconstructive surgery; the caecum, ascending colon, hepatic flexure and part of the transverse colon were mobilized and taken into the chest and neck subcutaneously. The caecum was anastomosed with the upper end of the oesophagus in the left side of the neck and the transverse colon with the stomach leaving the strictured oesophagus in situ in the thorax. The continuity of the gut was established by anastamosing the end of the ileum with the distal part of the transverse colon. The subcutaneous colon transplant was easily palpable under the skin of the chest wall and neck. Two years after the operation, that is about 3 years after the ingestion of the acid, he noticed drying and scaling of the skin of the trunk and limbs and also thickening of the palms and soles. He had been an alcoholic from his adolescence, but his appetite was good.

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Family history

He was born of non-consanguineous parents and his only sibling, a sister, was normal. His parents had some thickening of the soles of the type seen in people who walk barefooted. There was no other abnormality.

On examination

He had scars on his face due to the acid burns and scars on the front of the chest due to the subcutaneous colon transplant. The skin was generally dry and scaly and the palms and soles were thickened. There was no hepato- or splenomegaly and no lymphadenopathy.

Investigations

Routine investigations were within normal limits and the barium meal showed the colonic by-pass of the oesophagus with filling of the stomach. A biopsy from the ichthyotic area was reported as showing hyperkeratosis with some keratotic plugging. The granular layer was thin. There was atrophy of the rest of the epidermis. The dermis showed a mild perivascular infiltrate. The appearances were compatible with a diagnosis of ichthyosis vulgaris.

A biopsy from the palm showed a markedly thickened stratum corneum with a well formed granular layer. There was moderate acanthosis and the dermis showed some scattered cells. The sweat glands were within normal limits.

DISCUSSION

Tylosis of the late onset type may be related to the functional state of the oesophagus. In Shine & Allison's (1966) case the chronology was of dysphagia since the age of 18 months, followed by tylosis in adolescence and a cancer of the oesophagus when he was 61-years-old. In our case the tylosis was seen in a 23-year-old male following a surgical reconstruction of the oesophagus for a post-corrosive stricture. The chronological sequence of dysphagia followed by tylosis is therefore similar to that of Shine & Allison's (1966) case. In their patient, however, a carcinoma developed some years later at the site of the peptic stricture. Our patient at present has no evidence clinically of a carcinoma of the oesophagus and there is no way of determining the state of his oesophagus in the thorax other than by surgical inspection. However a post-peptic stricture or a post-caustic stricture may develop a carcinoma later (Clavell, 1960; Baret, 1960; Dubecz *et al.*, 1960 as quoted by Shine & Allison 1966).

The integument in our man of 23 years was completely normal until the colonic by-pass was performed for the stricture. The possibility of his developing tylosis about this age is unlikely as members of his family were all normal; the thickening of the soles in his parents was the normal thickening seen in bare footed individuals in the tropics.

In the light of the observations of an abnormal functional state of the oesophagus preceding tylosis of the late onset type, it would be interesting to know if any of Howel-Evans' (1958) series of patients with the late onset type of tylosis, who subsequently developed carcinoma of the oesophagus, had any symptoms related to the oesophagus prior to the tylosis. The carcinoma of the oesophagus in Howel-Evans' patients occured at an earlier age than is usual for cancer of the oesophagus, but theirs was a genetically determined disorder, whereas in our case the tylosis followed injury to the oesophagus.

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Comment

MEASUREMENT OF SENSORY CHRONAXIE AS A DIAGNOSTIC PROCEDURE IN SCLERODERMA

A possible relationship between scleroderma and the nervous system was suggested by several authors who called attention to the neurotrophic character of the skin lesions and such accompanying disorders as Raynaud's phenomenon (Hutchinson, 1896; Cassirer, 1912, 1935).

Neurohistological studies have demonstrated abnormalities in the nervous network of the skin both in the lesions and in apparently normal skin (John, 1950; Ormea, 1952, 1958; Coronini *et al.*, 1960; Thies, 1960; Pawlowski, 1963). Changes in the nervous network in other organs have been observed both in progressive systemic sclerosis (PSS) and in localized scleroderma (Pawlowski, 1963). These findings are a characteristic feature of systemic and localized scleroderma, which distinguish it from all scleroderma-like diseases studied so far. Neurohistological abnormalities in the connective tissue stroma (John, 1950; Ormea, 1952; Pawlowski, 1963) and around blood vessels (Kamiński, 1972) provide evidence in support of a pathogenic significance of the deranged nervous system.

Further evidence is provided by the highly characteristic prolongation of sensory chronaxie in apparently healthy skin in all cases of progressive systemic sclerosis and localized scleroderma. This phenomenon was noted incidentally in the early fifties, since when we have carried out our studies. We have found this method very useful, and regard it as one of the most reliable diagnostic criteria of the disease. Studies have been performed on 300 cases of PSS, on 280 cases of localized scleroderma, on about 600 control cases of various skin diseases, and on 248 cases of scleroderma-like conditions.

'Chronaxie' of a nerve designates the minimum pulse duration using a pulse height twice the rheobase. The rheobase is the minimum value of electric potential below which the nerve does not respond to stimulation. The normal values for sensory chronaxie range from 0·1 to 0·6 ms (Bourgnignon, 1923; Babkowa, 1954; Bubnow, 1954). Only prolongation of sensory chronaxe is of practical significance. This may be found in different types of skin lesions, and its degree depends on the character and depth of the pathological changes. The chronaxie indices vary accordingly, and are of no diagnostic importance. Chronaxie in the uninvolved skin is invariably normal.

Our first experiments were carried out using the apparatus described by Bourguignon (1923); in further studies our own apparatus was used (for technical aspects—see Dobrzyński, Szczepański & Mika, 1965; Dobrzyński, 1974). Modern equipment produced in various countries (Germany, Switzerland and others) was not suitable for the measurement of sensory chronaxie. Of basic importance for this measurement are low internal resistance of the pulse sources as compared to the resistance of the skin (modern equipment has a high internal resistance) and random production of pulses to avoid an adaptation effect (whereas in modern equipment they are repeated constantly). The electrodes should have a low resistance. The size of the active electrode should not exceed 1–2 mm; the silent electrode may be much larger, about 2 cm in diameter.* Both electrodes are moistened in saline before use, to ensure good electrical contact with the skin.

The patient provides the information about the response of his nervous system to the electrical pulse (Jablonska, 1974). Therefore the measurement must be carried out with special care and the patient should be well informed about the character and intensity of the threshold effect, and be accustomed to the examination. Chronaxie measurement cannot be carried out effectively in unconscious patients or in small children, who are unable to distinguish very weak threshold sensations. In hysterical persons the responses should be verified by tests with the current switched off.

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* Readers interested in the technical problems concerning the design and construction of the apparatus used by us, will be sent a description on request.

Studies in neurological and neurosurgical disorders

Various neurological (103 patients) and neurosurgical diseases (ninety-two patients) were studied to determine the level at which injury of the nervous system produces a prolongation of sensory chronaxie in the skin (Lukasiak, Bacia & Formas, 1961; Jablonska, 1963).

Injuries and tumours of the brain, even when associated with paresis, have no effect on sensory chronaxie. In cases of spinal tumours, of herniation of the nucleus pulposus, or of spinal trauma, sensory chronaxie was prolonged in the segment supplied by the respective roots.

Most interesting findings have been noted after sympathectomy (nineteen cases were examined). This was invariably followed by a prolongation of chronaxie in the corresponding limb, limited to the area innervated by the excised autonomic ganglion. In contrast, the rheobase, i.e. sensory threshold, was lowered. The prolongation of chronaxie was approximately parallel to the increase in electrical resistance of the skin in the extremity, and persisted for several weeks or months.

Peripheral nerve injuries produced variable prolongation of chronaxie only in areas of anaesthesia. The degree of prolongation was not proportional to the disorder of sensation and in some cases, in spite of hypoaesthesia, chronaxie remained normal. The greatest prolongation of chronaxie was noted after injuries of the brachial plexus associated with damage to the stellate ganglion, and after severance of the median nerve, which conducts the largest number of sympathetic fibres.

In no neurosurgical diseases was there a prolongation of sensory chronaxie in the entire skin.

These studies are in favour of a relationship between the prolonged sensory chronaxie and the autonomic nervous system, although some influence of the cerebrospinal system, especially nerve roots and peripheral nerves, cannot be excluded. Chronaxie seems to be independent of the higher levels of the cerebrospinal system.

Among the neurological diseases examined, prolongation of sensory chronaxie in the entire skin was found only in tabes dorsalis (Jablonska & Szczepański, 1965). In fifty-six of sixty cases studied chronaxie was slightly prolonged, usually just above the upper normal level. Chronaxie was normal only in abortive cases with no clear-cut clinical symptoms and with negative serology. The degree of prolongation of chronaxie was not related to the progress of the disease or to any detectable derangement of superficial sensation. The reason for this is not clear, but it is in accordance with the delayed conduction of sensory impulses, which is a characteristic feature of tabes.

Despite the impaired sensation in syringomyelia, of which eight cases were examined, chronaxie was normal except in areas with marked trophic disorders; the rheobase was markedly prolonged. In areas of complete anaesthesia neither the rheobase nor the chronaxie can be determined. Normal chronaxie in syringomyelia indicates that it is independent of the sensory nerves conducting pain impulses.

Influence of drugs on the nervous system (Jablonska, 1963)

The starting point was the surprising finding that sympathectomy is followed by prolongation of sensory chronaxie. Similar prolongation of chronaxie, confined to one limb, was seen after lumbar sympathetic block. Prolongation of chronaxie of a similar degree may be obtained in the entire skin after intramuscular injection of procaine (2–4 ml of a 4% solution), and lasts up to 6 h. Chronaxie is considerably prolonged in the entire skin after administration of ganglion blocking agents (azamethonium and hexamethonium) as well as after central sympatholytic drugs (chlorpromazine). The rheobase remains unchanged. These studies indicate a role of the autonomic ganglia and of the higher autonomic centres in the prolongation of chronaxie.

The sympathomimetic drug adrenaline slightly lowers chronaxie, and has no effect on the rheobase. Parasympathomimetic drugs have no effect on either chronaxie or rheobase. Analgesic drugs, acting on the cerebro-spinal system, do not affect chronaxie but raise the rheobase. Barbiturates produce prolongation of chronaxie and rheobase, probably acting on the higher levels of the cerebro-spinal and autonomic nervous systems. Tranquillizers like hydroxyzine have no influence on either chronaxie or rheobase. They probably act on the highest cortical centres which govern psychic reactions.

It is concluded that cutaneous sensory chronaxie, in spite of its relation to the sensory unit, depends on the autonomic nervous system, whereas the rheobase, or sensory threshold, depends on the cerebro-spinal system. The only neurological disease in which the chronaxie is prolonged in the entire skin is tabes dorsalis. We feel that too little attention has been accorded to autonomic disturbances in this disease.

In scleroderma, ganglion blocking agents and chlorpromazine have no effect on sensory chronaxie in PSS, or prolong it to a minimal degree both in the lesions and in the apparently uninvolved skin. In localized scleroderma there is a slight prolongation in the lesions as well as in the healthy skin. It seems that in PSS the autonomic nervous system has lost its ability to respond to stimuli, whereas in localized scleroderma this ability is markedly reduced.

Diagnostic and prognostic significance of prolonged chronaxie

Apart from scleroderma, prolonged chronaxie in apparently healthy skin does not occur in any other disease (excluding tabes dorsalis), and is therefore diagnostically significant, especially in differentiating scleroderma from scleroderma-like conditions, e.g. scleroedema of Buschke, sclerodermiform dermatomyositis, rheumatoid arthritis with trophic cutaneous changes.

It is also of importance in assessing PSS with no or very slight cutaneous involvement, and in cases which do not fulfil the clinical criteria of PSS.

In our series of 1475 cases, normal chronaxie was found in 397 cases diagnosed clinically as PSS; in follow-up studies these were proved not to have the disease. Normal chronaxie was found in seven patients suffering from chronic Raynaud's disease with trophic induration of the hands, but with no cutaneous lesions typical of PSS and with no internal involvement. It is possible that in some of these patients PSS will develop in the future.

Periodically repeated examinations are important in uncharacteristic cases, because even a slight but increasing prolongation of chronaxie may be a sign of beginning acrosclerosis for example in cases transitional between chronic Raynaud's disease and early acrosclerosis. On the other hand, slightly prolonged chronaxie remaining unchanged for several months or years indicates a mild or abortive course of acrosclerosis.

Decreasing values of chronaxie are a favourable prognostic sign. Sensory chronaxie in the uninvolved skin is an index of activity of the disease. The degree of prolongation of chronaxie in the normal skin reflects whether the morbid process has a tendency to spread, and is not at all or only slightly influenced by the extent of the lesions. Improvement is attended by shortening of chronaxie both in lesions and—which is much more important—also in apparently normal skin. Rise of chronaxie values after a period of decline predicts a possible relapse in spite of subjective and objective improvement. Consistently low values indicate that the disease is inactive.

When normal chronaxie is restored in the seemingly unchanged skin, a cure may be assumed. In no case of PSS have we noted normal chronaxie restored, although repeatedly slightly prolonged values have been found in some stationary or abortive cases.

In localized scleroderma, chronaxie returned to normal in forty-eight of 253 cases, and these cases may be regarded as cured. Full recovery usually takes years, depending on the extent of lesions and the activity of the disease. Repeated examinations in localized scleroderma are of prognostic significance.

Prolongation of chronaxie in seemingly unchanged skin occurs exclusively in PSS and localized scleroderma. This indicates that they are closely related, irrespective of marked clinical differences. Atrophoderma of Pasini–Pierini seems to be a special form of abortive morphoea. The sensory chronaxie does not exceed the upper normal limit but studies with ganglion blocking agents show some abnormal reactions (Jablonska, 1974). Studies on chronaxie favour its separation as an entity within groups of abortive localized scleroderma.

Summing up, it should be stressed that measurement of sensory chronaxie is of great diagnostic value in both systemic and localized varieties of scleroderma.

The shortcomings of the method consist in the subjective character of the patient's perception. A special, although rather simple, equipment is needed.

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Correspondence

ON THE CELL CYCLE OF PSORIASIS

SIR, The recent paper by Goodwin, Hamilton & Fry (1974) on the cell cycle in psoriasis has confirmed our earlier observations on the duration of DNA synthesis (Weinstein & Frost, 1968) but conflicts with some of our other kinetic data. It is the purpose of this letter to point out the discrepancies of these two reports; to explain the scientific reasoning that supports our measurements of the cell cycle; and to point out additional data that confirm our conclusions on the psoriatic cell cycle.

The principal difference in the two reports lies in the labelling index (L.I.) determined for the germinative cell population of the psoriatic epidermis (labelled DNA cells/total germinative cells). We obtained a L.I. of 22·7% ± 2·4 (s.d.) while Goodwin *et al.* (1974) obtained half that value with a L.I. of 11·0% ± 1·7 (s.d.). The first reason for this difference is the use of the factor (1·4 × observed L.I. = true L.I.) in our study to compensate for the weak energy of tritium, limiting beta emissions to 1·5–2·0 microns. A correction factor of 1·4 is used based on the experimental studies of Iversen & Evensen (1962) and the earlier observations of Quastler & Sherman (1959). The factor can be derived from the calculations that in 6 μm thick histological sections, which cut through cells at variable levels, thirty out of every 100 visible nuclei lie more than 2 μ beneath the surface of the tissue cut. Although these lower nuclei are visible microscopically, any tritium label contained in them does not have the energy to reach the photographic emulsion layered on the surface of the section and thus would not be recorded as labelled cells. The factor of 1·4 is used to compensate for this technical difficulty and raises the numerator of the L.I. fraction N_s/N_{gc}, where N_s is the number of labelled cells and N_{gc} is the number of germinative cells. Unfortunately it is not technically feasible to cut human skin specimens at a thickness of 2 μ for autoradiographic studies.

Goodwin *et al.* (1974) actually imply the validity of such a factor since they, like us and others, are unable to show a labelled mitosis curve reach above 70–80% because 'theoretically there may be some labelled nuclei deep in the section that fail to expose the photographic emulsion' (Goodwin *et al.*, 1974). If we use the factor of 1·4 for their data it raises their L.I. to 15·4% which brings the two sets of data closer together. Considering further the error of 10–15% as seen in the standard deviations from both studies there is almost statistical overlap. Suffice it to say that this point remains definitively unresolved but further evidence below indirectly supports its validity.

The second major variable for computing the labelling index is the determination of the denominator of N_s/N_{gc}, or the germinative cell population. Two problems can occur here. First, several reports have indicated that psoriatic proliferative cells are in the lower three epidermal rows. However, this is only so in the bottom of the acanthotic rete, whereas, along the sides of the rete and in the thinner areas of epidermis proliferative cells appear to be only one row deep (Weinstein, 1971). Thus, if N_{gc} is not scrupulously defined during the counting procedure, differentiated cells may be included in the count, thus elevating N_{gc} and decreasing the labelling index.

Secondly, counting cells directly under microscopic vision increases the error of N_{gc} by including cells that are underneath the superficial plane of cells when focusing up and down. In this manner it is possible to find portions of two cells occupying the same vertical column. In view of the two above variables, we determined the N_{gc} by counting cells from photomicrographs focused at the plane of the labelled cells. By this technique we could accurately count by a check-off system on the enlarged photomicrograph all visible nuclei, labelled or not, that occupied space at that plane of the section. While nuclei at a depth below 2 μm were still visible we were not counting additional nuclei that lay underneath the upper level nuclei.

Setting aside technical differences for the moment let us examine the turnover time of 120 h for the total epidermal compartment beneath the keratin in psoriasis found by Goodwin *et al.* (1974). They calculated a germinative cell cycle of 91 h and thus the difference of 29 h must be their transit (or turnover) time of cells in the differentiated cell compartment (stratum spinosum). This would mean that for every four cells in the viable epidermis, three would be germinative cells with only one differentiated cell to maintain the ratio of $N_{gc}/T_{gc} = N_t/T_t$ described in their paper. (N_t = total number of epidermal cells (4); T_t = turnover time for epidermis (120 h); $N_{gc} = 3$; and $T_{gc} = 91$ h.) We have counted the number of cells in both compartments of the psoriatic epidermis and found that the ratio between the proliferative and differentiated cell compartments is 100:76 or almost 1:1, much different from Goodwin's 3:1 (Weinstein & Housman, 1972). Thus it would appear that Goodwin's germinative cell compartment, N_{gc} is falsely high by including differentiated cells in this compartment.

Further definitive proof of our conclusions was recently presented by Grove *et al.* (1974). They used cytophotometric studies to evaluate the cell cycle in psoriasis. With this technique the proportion of cells in the G_1, S and G_2 phases can be measured by examining the cellular DNA content after Feulgen staining with a microdensitometer. Their results indicate that the ratio of $G_1:S:G_2$ cells is 5:2:1 which almost identically compares to the ratio of 6:2:1 based on our cell cycle compartment times of 25:8·5:4h. In comparison the ratio from Goodwin's data would be 15:2:1, once again indicating a three fold higher count of the proliferative compartment leading to a lower labelling index and a prolonged psoriatic cell cycle.

The final evidence we suggest in support of the psoriatic cell cycle of 37 h is the experiment in which the entire psoriatic germinative cell population was synchronized into the S phase by administering methotrexate intradermally over a 36 h period. At that point the injection of thymidine -^{3}H labelled the entire proliferative pool of cells as predicted by a cell cycle of 37 h (Weinstein, 1971).

In summary we feel that the discrepancies may reflect slightly different experimental approaches that can substantially alter the final results. Further support for our published results is provided from three different experimental investigations.

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A reply

SIR, We feel that these criticisms of our paper merit a reply. A number of the technical points concerning the evaluation of the labelling index (L.I.) have already been fully discussed in the paper.

The convention of multiplying the L.I. by a correction factor of 1·4 is no longer generally observed by cell biologists. Mention has already been made in the paper of the experiment carried out by Gelfant (1961) who in his studies of mouse epidermis showed that the labelling indices obtained from 2 μ and from 7 μ thickness histological sections were the same. Therefore when using 5 μ sections this correction is unnecessary.

Although we stated that 'theoretically there may be some labelled nuclei deep in the section that fail to expose the photographic emulsion' in the next sentence we state that 'we do not believe it to be a significant factor; certainly not of the order to significantly alter our results'.

The definition of the germinative cell population N_{gc} cannot be scrupulously defined as there is no specific stain for these cells. We have clearly stated the zone of cells that we define as germinative and admit that it is a subjective observation. However, we cannot accept that the photo-micrograph is a more accurate method of defining cells than direct viewing under the microscope.

The turnover time for the total viable epidermal compartment is fairly easily obtained by counting the total number of cells, as described in the paper. This does away with the need to differentiate between cell types as proliferating and differentiating cells are included.

We cannot comment on the cytophotometric studies of Grove *et al.* (1974) as we do not have the details of the data and methods.

The observations on thymidine—^{3}H labelling after methotrexate (Weinstein, 1971) do not, we feel, provide any conclusive proof of the cell cycle time.

The actions of this drug in psoriasis are not yet fully understood, and certainly the drug appears to have other actions than blocking DNA synthesis.

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MYCETOMA AND *TRICHOPHYTON RUBRUM*

SIR, I recently read the publication, 'Mycetoma formation in *Trichophyton rubrum* infection', by Burgoon *et al.* (1974). The case presented by these authors closely resembles a patient described by us (Thorne & Fusaro, 1971). Our patient also developed small lumps on the lower extremities that slowly enlarged and discharged pus. Of note, our patient was also taking prednisone, 30 mg a day, at the time of the onset of her *Trichophyton rubrum* abscesses. We were fortunate in our case to be able to demonstrate the organism by culture and by direct microscopy.

I believe these two cases support the concept that an innocuous organism, such as *Trichophyton rubrum,* can cause a spectrum of diseases all the way from a chronic scaling dermatosis of the foot to subcutaneous abscess and even mycetoma abscesses. When patients taking steroids, even in low doses, develop abscesses, we should always look for an organism like *T. rubrum* which may respond to griseofulvin.

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TRACTION ALOPECIA IN SIKH BOYS

SIR, Traction alopecia due to slow continuous traction is well-known in girls with pony-tail hairstyles, in those who use rollers to curl their hair, and in negroes who plait their kinky hair to straighten it.

A similar traction alopecia is seen in Sikh boys. In the Sikh religion males do not cut their hair. To keep their long hair well groomed and kempt, the hair is made into a plait, twisted and put up on top of the head, where it is kept for 12 to 24 h before recombing (Fig. 1). This is a universal hairstyle in all Sikh male children. They try to twist the hair so tight that while playing it does not come undone. As in

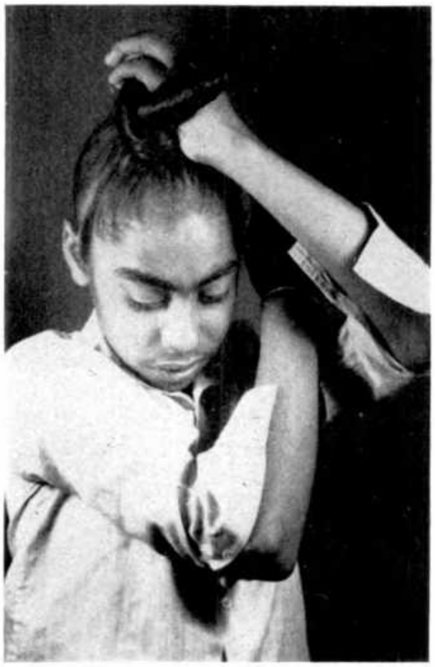

FIGURE 1.

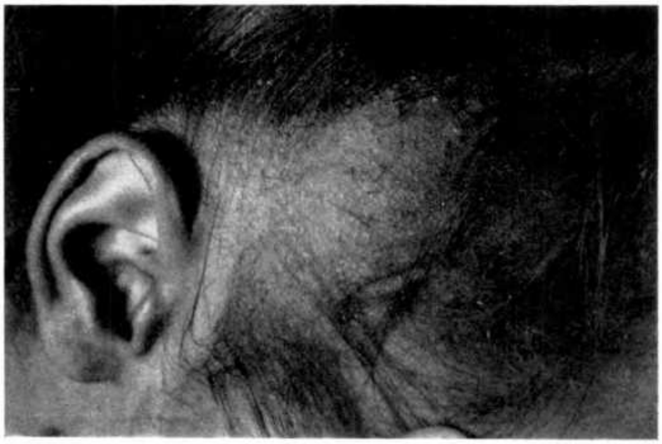

FIGURE 2. Traction alopecia of scalp parietal region

other types of traction alopecia, the periphery of the scalp, especially in the frontal and parietal regions, is chiefly involved (Fig. 2).

Treatment is to make the hair-do looser. In early and mild cases full regrowth may occur, but in longstanding cases this may be incomplete.

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CIRCULAR REGROWTH IN ALOPECIA AREATA

SIR, Treatment of alopecia areata with corticosteroids, both by local application and by intradermal injection, has been practised for many years. Intralesional injections appear to be the most effective, but there is no doubt that the more potent topical preparations can promote some degree of regrowth, although relapse is common with both methods (Abell & Munro, 1973; Leyden & Kligman, 1972). In alopecia areata a centrifugal wave of hair follicle damage is followed by a regrowth of new hairs (Eckert, Church & Ebling, 1968).

We have recently seen eight patients in whom the regrowth has taken a peculiarly circular form. All of these patients had been using fluorinated corticosteroid applications (Table 1). Four patients admitted that they applied the preparation by rubbing vigorously in a circular manner.

TABLE 1. Preparations used and duration of treatment

Case	Sex	Age	Steroid preparation	Duration of use when regrowth noted (weeks)	Circular rubbing
1	F	38	Synalar Forte	10	Denied
2	F	13	Betnovate Scalp Application	8	Admitted
3	F	21	Synalar Forte	5	Denied
4	F	38	Synalar Forte	11	Admitted
5	M	25	Synalar Forte	8	Possible
6	F	24	Synalar Forte	16	Possible
7	M	49	Synalar Forte	16	Admitted
8	M	39	Synalar Gel	6	Admitted

All of the lesions subsequently regrew hair in the central area and eventually disappeared (Figs 1, 2 and 3).

We wonder if this peculiar regrowth pattern is an artefact due to the unconscious tendency to rub in a circular manner with maximum concentration at the edge of the lesion, or whether it is related to the ripple like spread of disease activity.

ACKNOWLEDGMENT

We would like to thank the photographic department of St John's Hospital for Diseases of the Skin for the illustrations.

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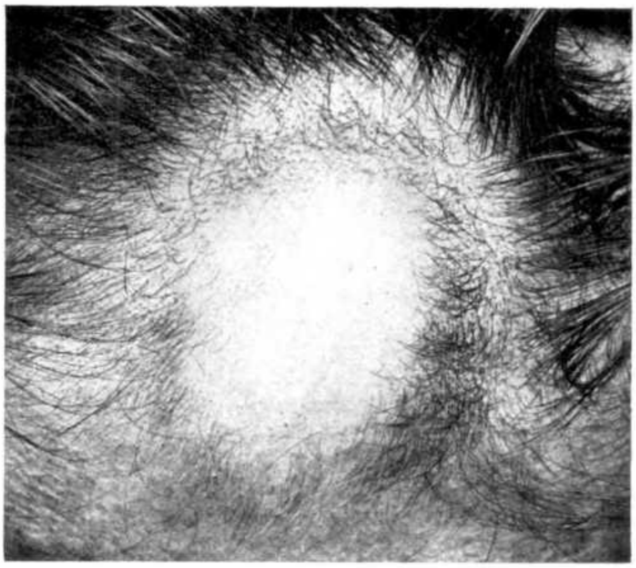

FIGURE 1. Patient No. 7. Circular regrowth in characteristic pattern after application of steroid for 16 weeks.

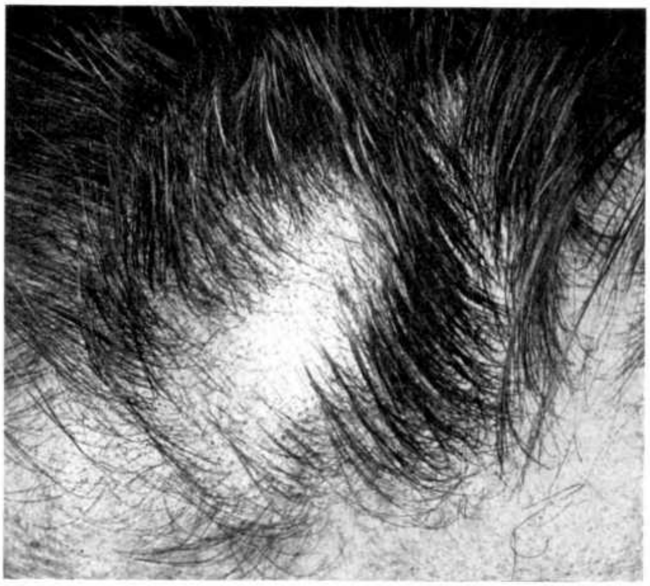

FIGURE 2. Same area as Fig. 1, 8 weeks later.

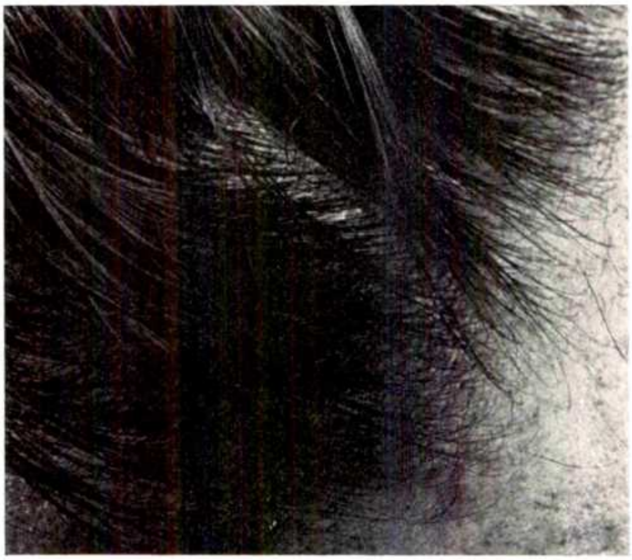

FIGURE 1. Same area as Fig. 1, 10 weeks later.

Book Reviews

Proceedings of the 14th International Congress of Dermatology, Padua/Venice, 22–27 May 1972. F.FLARER, F.SERRI and D.W.K.COTTON (Ed.) (1974) Amsterdam: Excerpta Medica. Pp. 1008, illustrated. Price £44.80

The proceedings of the Venice International Congress cover virtually all aspects of dermatology. They should therefore, and do, contain something of value for everyone, albeit somewhat diluted. The list of contents alone runs to 22 pages. The papers which succeed most are either those where a world authority highlights the salient problems of some larger problem, without attempting to cover the whole field, or the shorter papers which convey only a single simple message. For example, the review of immunotherapy of malignant melanoma by Kopf brings together material from many sources. It is now generally accepted that International Congresses are not the forum where new material is presented, and virtually nothing emerges that has not appeared in print elsewhere. However, few can be aware, for instance, of the distinctive acral psoriasiform eruption described by Bazex, Dupré and Christol, which so far has been associated with a systemic carcinoma as regularly as is acanthosis nigrigans. Many of the genodermatoses described are also likely to be unfamiliar.

The papers are all written in English, a remarkable tribute to somebody's industry. Some authors have been content to allow their original summary printed before the Congress to be published, and several have failed to provide even a key bibliography. The editors and publishers are to be congratulated on marshalling together so attractively such unwieldy material, much of which was perhaps more suitable for verbal than written presentation.

Most individual dermatologists will probably feel that the average amount of useful information per page makes this book too expensive to buy, but will nevertheless find that there is much of value in it and they will want access to it in libraries. It can be appreciated why the organizers of the next congress are apparently considering alternative ways of recording the proceedings.

R.H.CHAMPION

Lupus Erythematosus. A review of the current status of Discoid and Systemic Lupus Erythematosus and their variants. EDMUND L.DUBOIS (Ed.) (1974) second edition. Los Angeles: University of Southern California Press. pp. xv+798. Price $36.00.

The first edition of this excellent review was published in 1966. So much has been written about the subject since then that 300 pages and 1500 new references have been added in the second edition. The original chapters are retained but most have been supplemented by additional ones. There are two new chapters on the New Zealand mouse model and on coagulation defects.

An appendix contains an explanatory pamphlet for handing out to patients with lupus erythematosus. This is admirable for patients suffering from systemic lupus erythematosus but might scare patients with discoid lupus erythematosus whose risk of developing systemic lupus erythematosus is agreed to be less than 5%. If a pamphlet is required for patients with discoid lupus erythematosus it should be short and separate from that for systemic patients.

It is good to see the value of rest being mentioned in the management of systemic lupus erythematosus.

A book of this sort is invaluable to clinicians and research workers in the field and the editor and his eighteen contributors are to be congratulated on including references as recent as February, 1974. Further editions would be enhanced for the general physician and dermatologist if the editor, using his own enormous experience, would give an evaluation of the main developments at the end of each chapter.

N.R.ROWELL

Urticaria. ROBERT P.WARIN and ROBERT H.CHAMPION (1974) London: W.B.Saunders. Pp. 173 illustrated. Price £3.80.

The first volume in the new series 'Major Problems in Dermatology' edited by the indefatigable Arthur Rook, is a monograph on urticaria. It has been written by two acknowledged experts in this field, Dr Robert Warin of Bristol and Dr Champion of Cambridge. The authors have interested themselves in this subject over their professional life time and both have published widely on various aspects of urticaria and angio-oedema.

It is a beautifully produced little book of only 172 pages, it is light to hold and easy to read. It is divided into nine chapters and throughout the text is concise, the illustrations are quite adequate and the references, which are largely taken from English language journals, profuse.

The authors with their immense knowledge of the subject show an open mind and there is a startling lack of dogma. They give considerable space to older reports and concepts and often their own views are not forcibly expressed. However, it is with urticaria that an open mind must be maintained, for it is a baffling and complex subject which only now starts to emerge from the mists of folklore to enter a more respectable arena of scientific explanation and understanding.

All practising dermatologists will want to possess this book and it is no secret that I bought my own copy before being asked to review it! The index is comprehensive, e.g. onchocerciasis, onions, orange peel, ornithosis, osmylogenic urticaria, oxytocin! I recommend it without reservation, its price is only £3.80.

STEPHEN GOLD

The Herpes viruses. ALBERT S.KAPLAN (Ed.) (1973) New York/London: Academic Press Inc. Pp. 739, 91 figures, 49 tables. Price £14.90.

Infection with herpes simplex virus is probably the commonest infection of mankind. The herpes viruses have fascinated the virologists because of their capacity to enter into a latent state in hosts that have recovered from clinically apparent disease and perhaps later to cause recurrent infection. In addition, the diseases caused by these viruses, and their possible association with cancer, have stimulated a great interest in the group as a whole.

With increasingly sophisticated cell culture methods and animal techniques there has been a rapid accumulation of knowledge about the interaction of these viruses with their hosts at both the cellular and molecular levels. Thus there is now much more understanding on the replication of herpes viruses and some suggestion that certain members of this group may have oncogenic potential, and there is no doubt that the time is ripe for a comprehensive reference book on the herpes viruses.

The first part of the book covers both basic and clinical aspects of all herpes viruses. Thus classification, morphology, physico-chemical properties, replication, immunological relationships, interferon and interference as well as persistent and latent infection are covered in the first part of the book.

The second part of the book is more clinical and deals primarily with specific diseases of man and animals, and includes descriptions of the essential clinical features, pathogenesis, immunity and epidemiology, with four chapters devoted specifically to those herpes viruses linked with carcinogenesis. Finally, the last two chapters discuss molecular and clinical aspects of antiviral chemotherapy.

Twenty-six contributors, including Dr A.S.Kaplan (the Editor), have produced a timely, well balanced, comprehensive and scholarly reference book. There is no doubt that this large but concise volume will be very useful to many workers in many disciplines whether they be in research or clinical practice. One can only hope that Dr Kaplan, his co-authors and the publishers are planning the future editions that will be needed to keep us all informed of the many advances in our corporate knowledge of this fascinating and important group of viruses.

T.W.E.Robinson

British Journal of Dermatology (1975) **92,** 239.

Review

HAIR I

The clinical significance of abnormal hair-slope patterns—Trichoglyphics

Dermatologists commonly use the term hair pattern to describe the relative distribution over the body surface of terminal and vellus hairs, and those variations in pattern which are androgen-induced have been extensively investigated. The zoologist and the comparative anatomist apply the term to the distribution of hair tracts, which determine the direction of slope of hair follicles in various regions of the body. These hair-slope patterns have until recently attracted little interest among clinicians, but it is now evident that their further study may be rewarding.

Hair-slope patterns in man and various other animals were recorded by some of the great anatomists of the nineteenth century, and stimulated much speculation concerning their origin and significance. Walter Kidd (1853–1929), who was a successful general practitioner at Blackheath, near London, and an enthusiastic amateur geneticist, devoted many years to the detailed study of hair-slope patterns. He brought together much of the material in his earlier publications in a slim volume published in 1901 and entitled 'Use–inheritance illustrated by the direction of hair on the bodies of animals'. Two years later he published a much more comprehensive book on the same subject. As the title of the earlier book implies Kidd maintained a Lamarckian standpoint, for his ingenious explanations of species differences in pattern, in terms of their habits in grooming and in rain protection, depended on the inheritance of acquired characters.

In 1924 Bolk, the professor of anatomy at Amsterdam, published a report on the frontal hair-slope patterns in sixty human fetuses. He was impressed by their great variability. He dismissed Kidd's incrimination of external factors, but could only conclude rather lamely that 'hair slope is probably the expression of certain conditions of growth in the skin'. Surprisingly, perhaps, another distinguished anatomist, F. Wood Jones, then of Adelaide, came to Kidd's defence. He believed that in the primitive condition the hair sloped caudad and ventrad on the trunk, and post-axially and distally on the limbs. He thought, like Kidd, that this hypothetical primitive pattern had been modified by 'the actual purposive activities of the animal itself' (Wood Jones, 1924–25a, b). He supported his argument with a series of observations on hair-slope patterns in certain marsupials, in which he thought the method of grooming had determined the pattern. After examining Wood Jones' seductively convincing drawings, it has reluctantly to be admitted that the significance of hair-slope patterns was still obscure.

There were few clinical studies of hair-slope patterns. However Kiil (1948a) took a useful step forward when he classified the frontal hair-slope patterns in a Norwegian population into three types and showed that there was complete concordance in uni-ovular twins and 43% in binovular twins. Inheritance was apparently determined by autosomal dominant genes. Later Kiil (1948b) studied mental defectives in Connecticut. The same three principal patterns were observed as in the earlier investigation, but there was evidence of racial variation in the frequency of the various types. The subjects included sixty-six mongoloids, amongst whom one of the three patterns did not occur.

Recently important advances have been made by Smith & Gong (1974) of the Dysmorphology Unit of the University of Washington. These investigators studied hair-slope patterns in the scalp in normal fetuses and in those with disorders of early brain development, and human patterns were contrasted with those of other primates. As a result they found it possible to put forward the following hypothesis: the direction of the hair slope is secondary to the relative plane of growth–stretch exerted on the developing skin whilst the hair follicles are forming; thus aberrations in the growth and shape of the brain between approximately the 10th and 15th (probably the 10th and 12th) weeks of fetal life can give rise to aberrant hair-slope patterns. Voigt of Vienna in 1857 had put forward similar views on the factors determing the hair slope but had been unable to provide the evidence to support his hypothesis. The arguments of Smith & Gong are convincing and the clinical implications of their work are of interest, not least to the numerous dermatologists with enquiring minds who lack laboratory facilities or the capacity to use them. Recording hair-slope patterns presents no great practical difficulties, unless the hair is long.

To obtain further information on normal scalp hair-slope patterns Smith & Gong examined 200 white children and they described and illustrated their findings. There were no significant sex differences. In 95·5% there was a single parietal whorl. In patients with disorders of early brain development, the hair-slope patterns were abnormal. For example 85% of patients with primary microcephaly had abnormal patterns; 25% had no parietal whorl, a pattern previously described only in non-human primates.

Smith & Gong then extended their investigation to a number of well-defined dysmorphic syndromes, and in five of them they found abnormal patterns. The recording of what the authors suggest should be called trichoglyphics would appear to be a potentially profitable new approach to the study of many complex developmental syndromes, and usefully to complement the recording of dermatoglyphics.

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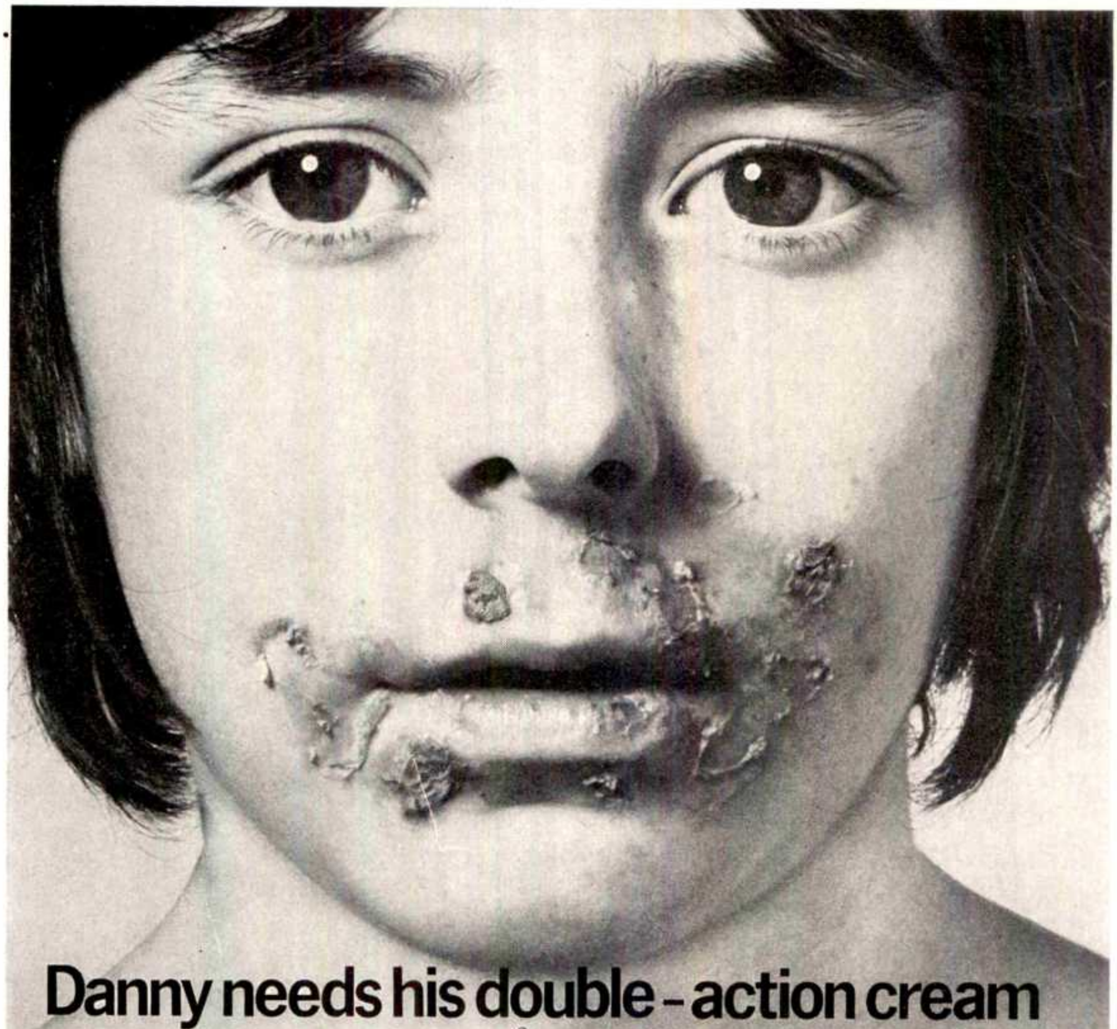
* As cited by Bolk (1923–4). It has not been possible to consult Voigt's monograph.



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Index Vol. 149, No. 2, 1974

Reymann, F. (Copenhagen): Treatment of Discoid Lupus Erythematosus with Betamesone–Valerate Cream 1%

MacKie, Rona M. (Glasgow): Sweet's Syndrome. A Further Case of Acute Febrile Neutrophilic Dermatosis

Remy, W.; Bockendahl, H., and Antoniadis, G. (Berlin): Comparative Studies of Rubella Antibodies in Psoriatic Patients Treated with Methotrexate

Pospíšil, L. and Kratochvil, F. (Brno): Vor- und Nachteile des Peroxidase-Tests mit *Treponema pallidum* in der Serodiagnostik von Syphilis im Vergleich mit dem FTA-ABS-Test
(Advantages and Disadvantages of the Peroxidase Test with *Treponema pallidum* in Diagnostics of Syphilis as Compared with the FTA-ABS Test)

Hagedorn, M.; Thomas, C. und Kannen, W. von (Freiburg i. Br.): Dermatofibrosarcoma protuberans mit Übergang in ein sogenanntes Fibrosarkom
(Dermatofibrosarcoma Protuberans with Transition into a Socalled Fibrosarcoma)

Björnberg, A. (Gothenburg): Low Alkali Resistance and Slow Alkali Neutralization. Characteristics of the Eczematous Subject?

Bruyn, C.H.M.M. de and Oei, T.L. (Nijmegen): Purine Phosphoribosyl Transferases in Human Hair Roots

Undeutsch, W. und Schieferstein, G. (Tübingen): Das Knoblauchzehenfibrom
(The Garlic-Clove Fibroma)

Borovanský, J. and Duchoň, J. (Prague): Chemical Composition of Hair Melanosomes

Christiansen, J.V.; Gadborg, E.; Ludvigsen, K.; Konstmann Meier, C.H.; Nørholm, A.; Osmundsen, P.E.; Pedersen, D.; Rasmussen, K.A.; Reiter, H.; Reymann, F.; Rosman, N.; Sylvest, B.; Unna, P.; Wehnert, R.; Aastrup, B.; Andersen, B., and Holm, P. (Aarhus/Copenhagen): Topical Vitamin A Acid (Airol®) and Systemic Oxytetracycline in the Treatment of Acne Vulgaris. A Controlled Clinical Trial

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F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

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Disposition of serotonin in the rat blood vessels and heart

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Actions of the sympathomimetic bronchodilator, trimetoquinol (AQL 208) on the cardiac, respiratory and skeletal muscle systems in the anaesthetized cat, and on cat isolated atrial and tracheal preparations

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Volume 1, Number 4, August 1974

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Heinz W. Kunz and Thomas J. Gill, III. The effects of antigenic isomerism and antigenic aggregation on the antibody response in inbred rats.

B. Cinader. Six years of the International Union of Immunological Societies.

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gram(s)	g	minute(s)	min	molar	mol/l
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milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
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British Journal of Dermatology, Volume 92, Number 2, February 1975

CONTENTS

AGE

Clinical and Laboratory Investigations

Langerhans Cells in Tissue Cultures of Guinea-pig Epidermal Cells 121
C. N. D. CRUICKSHANK AND J. R. COOPER

Patch Testing in the Rabbit using a Modified Human Patch Test Method—Application of Histological and Visual Assessment 131
A. J. INGRAM AND P. GRASSO

Photochemistry of Tetrachlorosalicylanilide and its Relevance to the Persistent Light Reactor 143
A. K. DAVIES, N. S. HILAL, J. F. MCKELLAR AND G. O. PHILLIPS

A Study of Physical Light Screening Agents 149
T. M. MacLEOD AND W. FRAIN-BELL

Immunoglobulins in the Skin in Dermatitis Herpetiformis and their Relevance in Diagnosis 157
P. P. SEAH AND LIONEL FRY

Contact Dermatitis in Animal Feed Mill Workers 167
D. BURROWS

Trichophyton Rubrum Infections According to Age, Anatomical Distribution and Sex 171
F. BLANK AND S. J. MANN

A Search for Microtubular Inclusions in Endothelial Cells in a Variety of Skin Diseases 175
R. F. MacADAM, J. M. VETTERS AND N. K. SAIKIA

Post-inflammatory Elastolysis and Cutis Laxa 183
A. R. VERHAGEN AND M. J. WOERDEMAN

Localized Heat Urticaria Treated by Inducing Tolerance to Heat 191
I. M. LEIGH AND C. A. RAMSAY

Pharmacology and Treatment

An Explanation for the 'Placebo' Effect of Bland Ointment Bases 195
S. TREE AND R. MARKS

Double-blind Controlled Crossover High-dose Study of Azaribine in Psoriasis 199
WILLIAM A. CRUTCHER AND SAMUEL L. MOSCHELLA

Case Reports

Juvenile Melanoma Disseminatum 207
JOHN CAPETANAKIS

A Fixed Drug Eruption due to Paracetamol 213
H. T. H. WILSON

The Smell of Cancer 215
KEITH LIDDELL AND JOHN WHITE

Tylosis Following Post-corrosive Stricture of the Oesophagus 219
A. S. THAMBIAH, P. YESUDIAN, S. M. AUGUSTINE, A. KAMALAM AND S. PREMALATHA

Comment

Measurement of Sensory Chronaxie as a Diagnostic Procedure in Scleroderma 223
STEFANIA JABLONSKA

Correspondence

On the Cell Cycle of Psoriasis 229
GERALD D. WEINSTEIN
Reply by PETER GOODWIN, SUSAN HAMILTON AND LIONEL FRY 230

Mycetoma and *Trichophyton Rubrum* 231
GEORGE THORNE

Traction Alopecia in Sikh Boys 232
GURMOHAN SINGH

Circular Regrowth in Alopecia Areata 233
R. S. H. TAN AND T. J. DELANEY

Book Reviews 235

Review

Hair. I. The Clinical Significance of Abnormal Hair-slope Patterns—Trichoglyphics 239
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References

1. Brit. J. Derm (in press)
2. Archs. Derm. **86**, 608, 1962
3. Brit. J. Derm. **90**, 197, 1974

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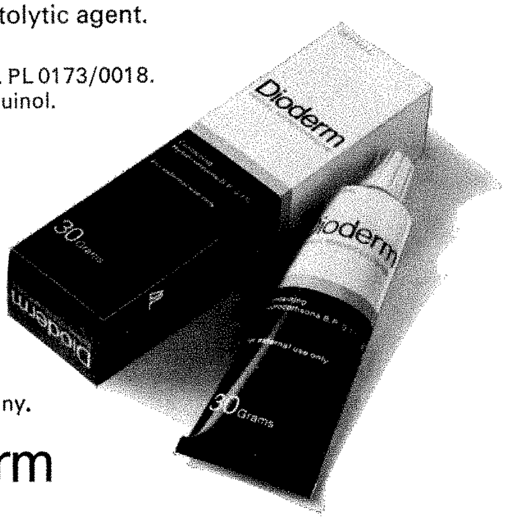

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1. Almeyda, J. and Burt, B.W., *Brit. J. Derm.* (To be published) 2. Harries, E. H. L. and Jacoby, R. H. (To be published)
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*Benzoyl peroxide gel in the treatment of Acne vulgaris
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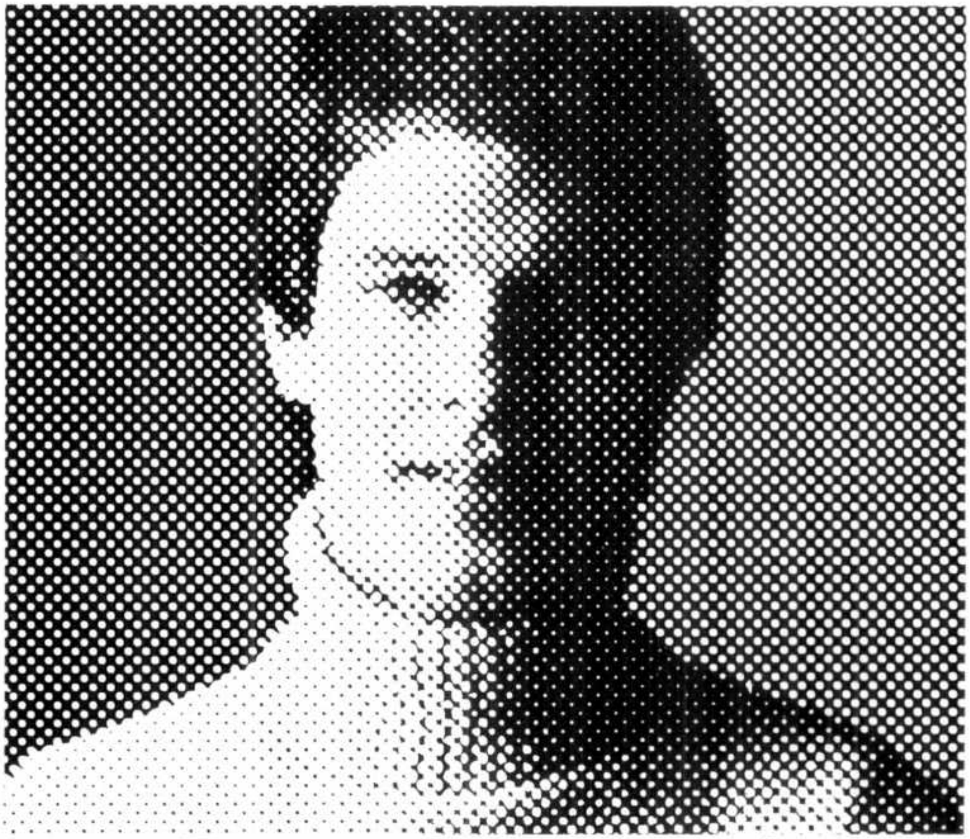

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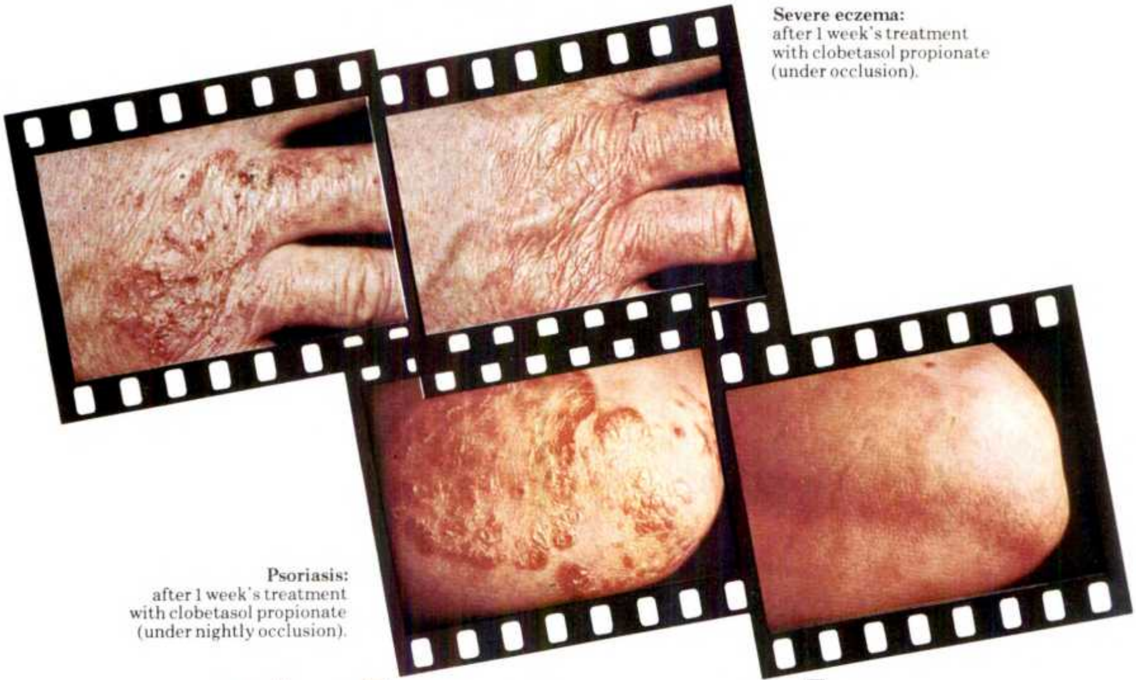

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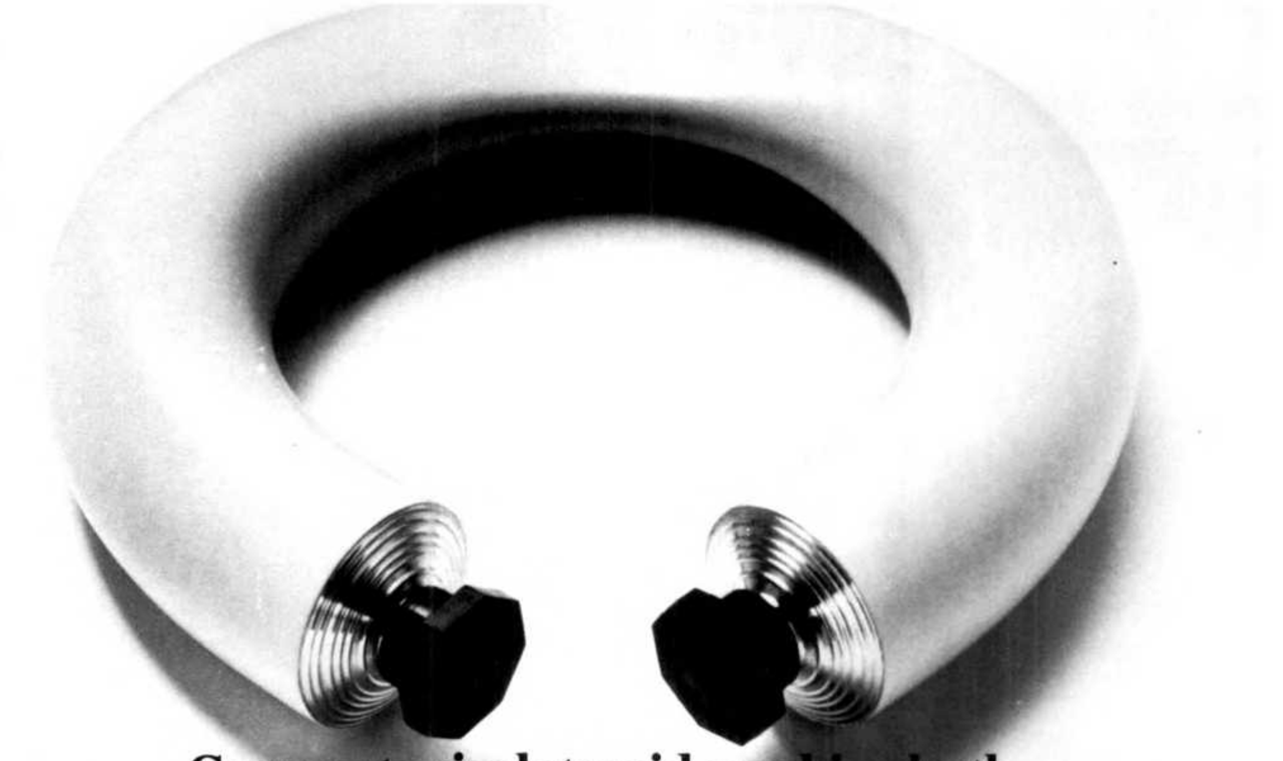

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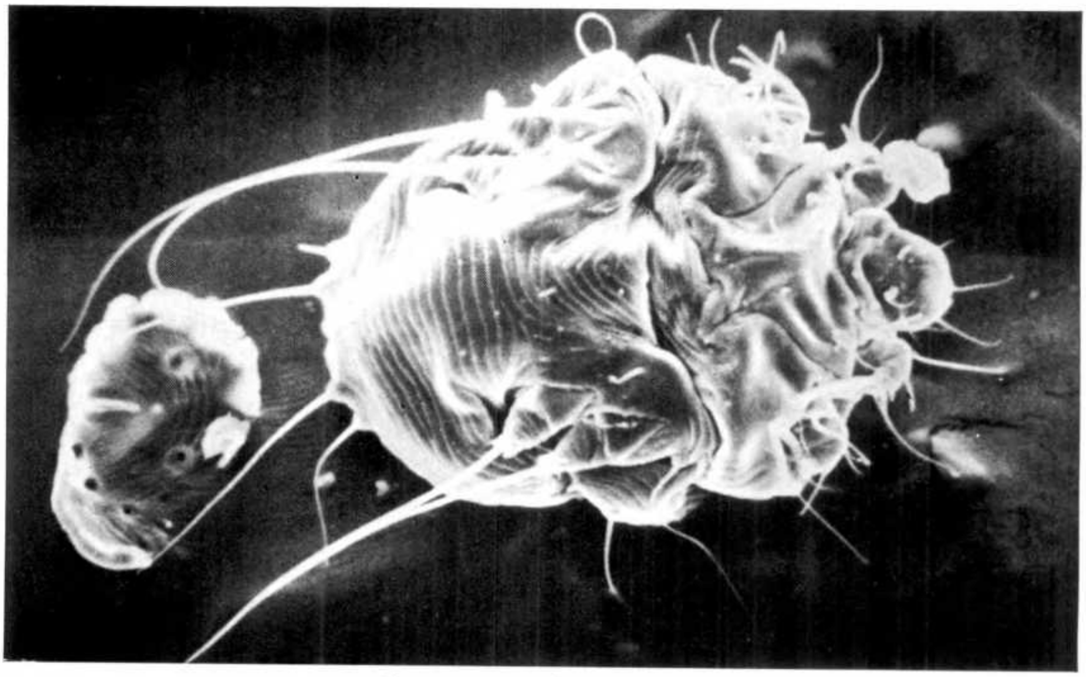

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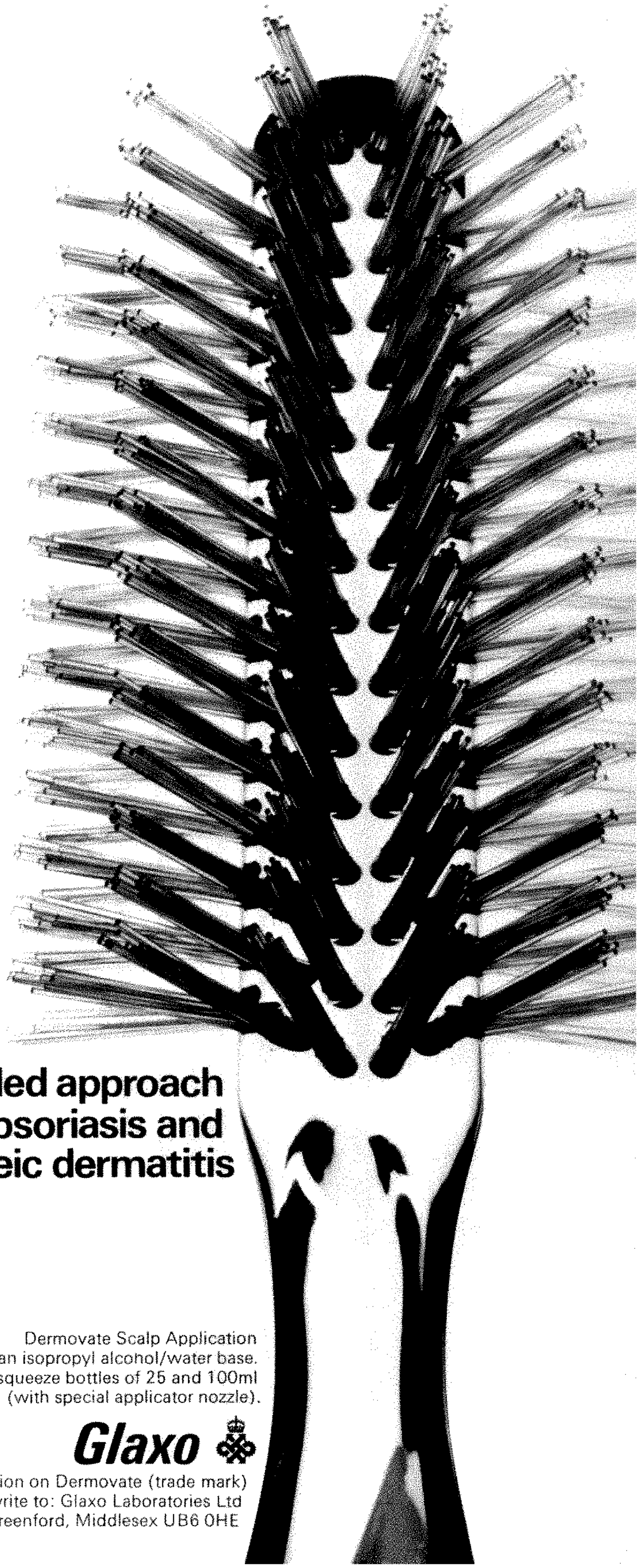

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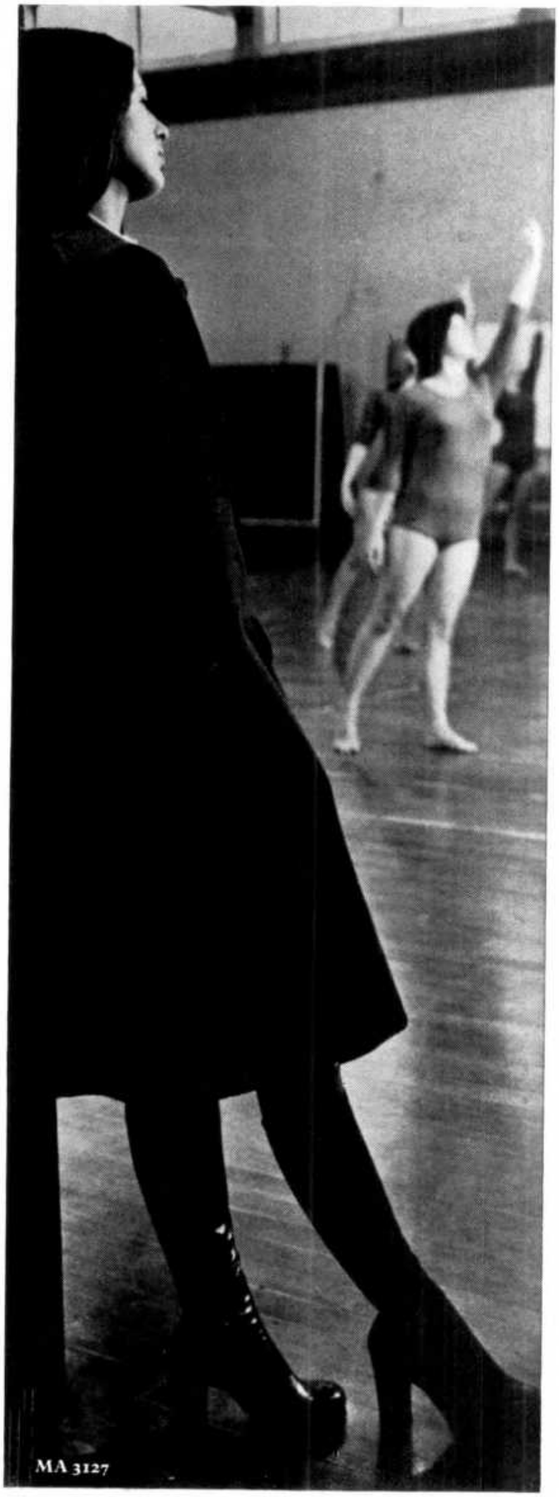

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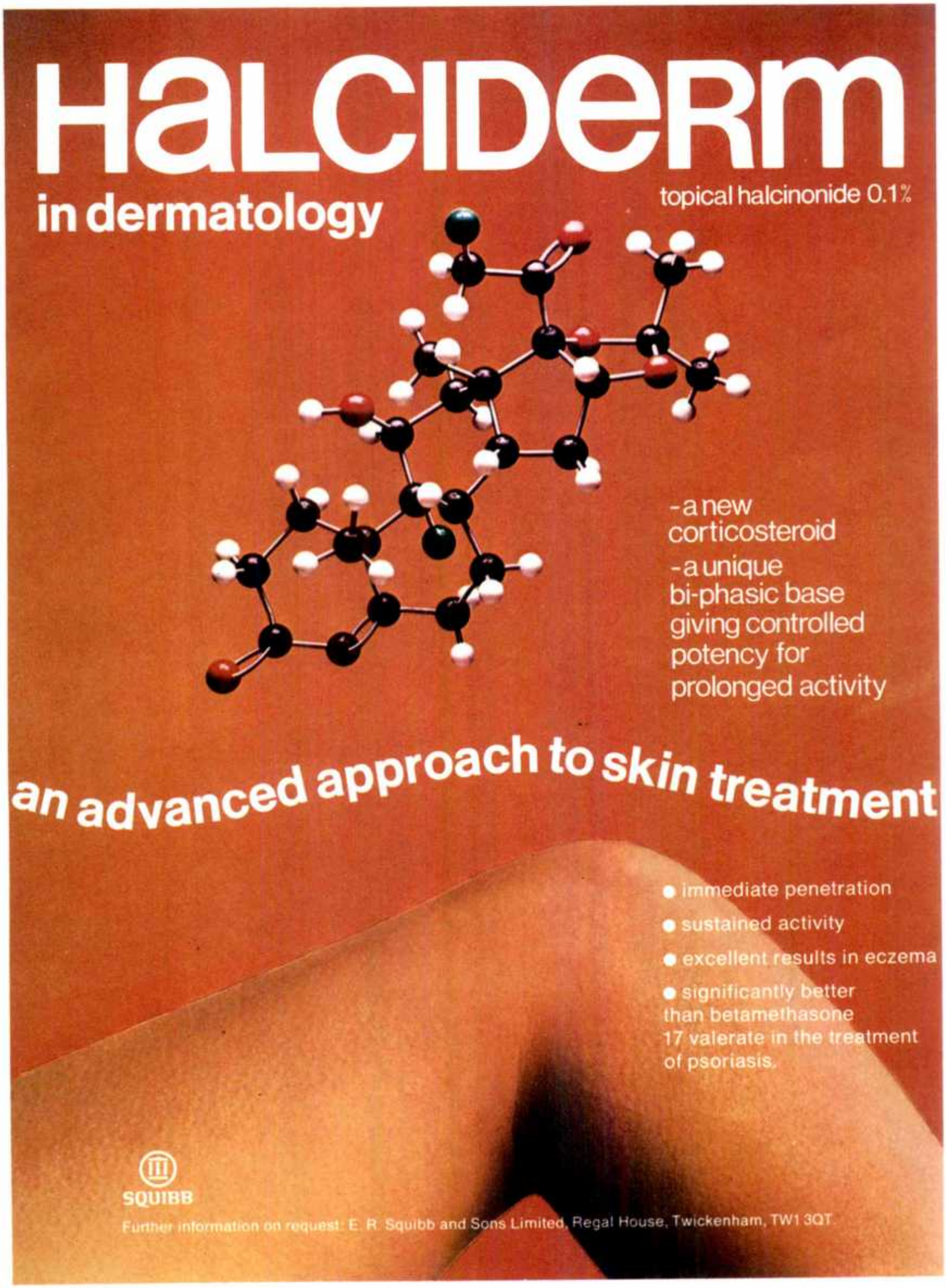

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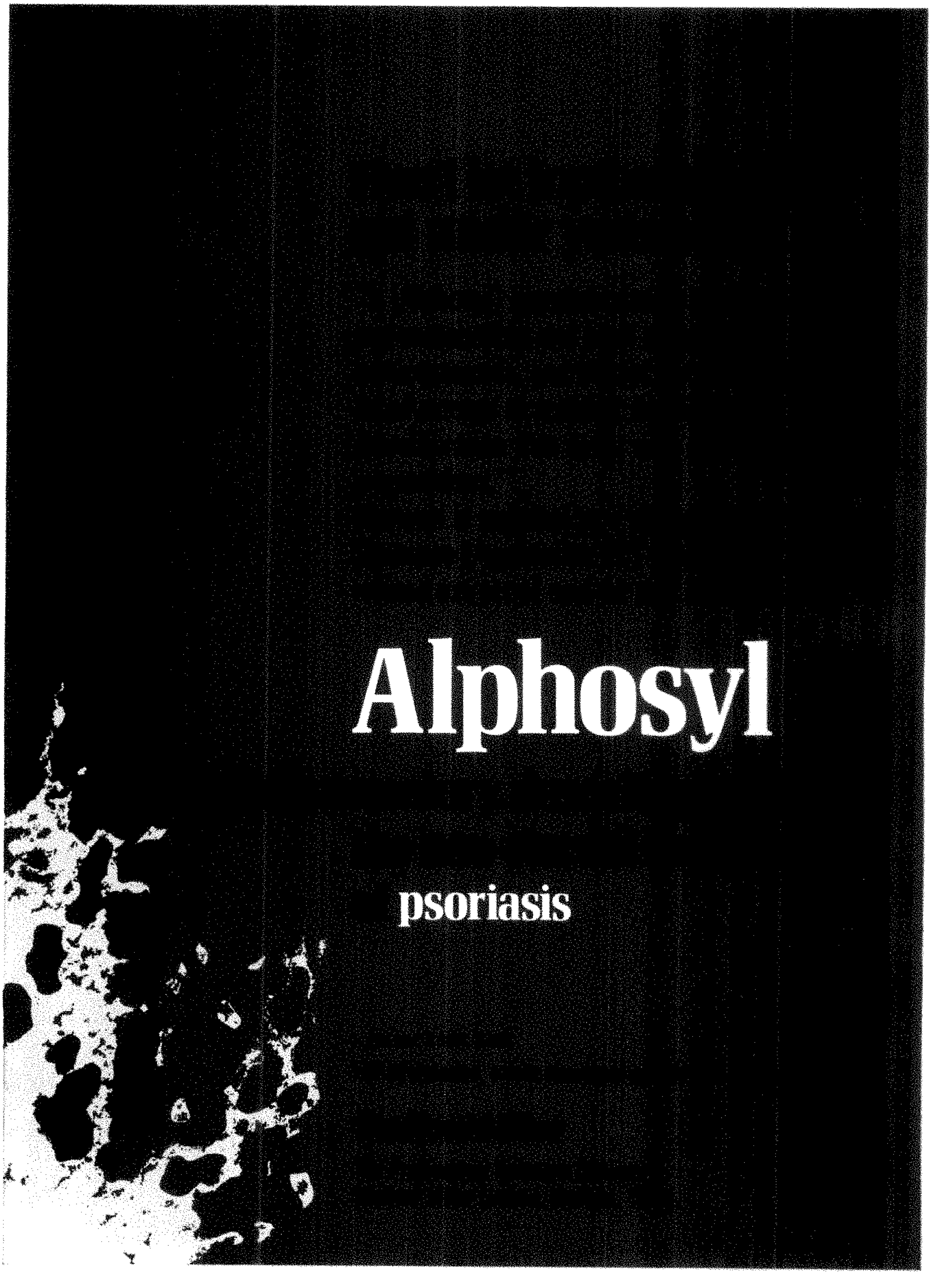
Alphosyl
psoriasis

British Journal of Dermatology (1975) **92**, 241.

Clinical and Laboratory Investigations

Micro-determination of cyclic AMP levels in human epidermis, dermis and hair follicles

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SUMMARY

In order to study the biological and possible pathological roles of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in the skin, it is mandatory to measure cyclic AMP in 50–100 μg of microdissected epidermis, dermis or appendages.

In the present study, we offer a method of extracting cyclic AMP from less than 100 μg of tissue, removing contaminating nucleotides and scaling down Gilman's method to fit the analysis of small amounts of tissue. Cyclic AMP levels in the dermis, epidermis, and hair follicles (bulbs) were approximately 1, 2 and 3·5 pmols/μg dry weight tissue respectively.

This procedure is applicable to the measurement of cyclic AMP levels in limited foci of healthy or diseased skin.

Although cyclic AMP levels have been measured in a wide variety of mammalian tissues, extremely limited data on skin are currently available. Voorhees *et al.* (1972, 1973) measured cyclic AMP levels in keratome slices of normal and psoriatic skin and reported that the psoriatic contained less cyclic AMP than uninvolved skin of the psoriatic patient. Härkönen, Hopsu-Havu & Raij (1974) also working with keratome slices reported, on the contrary, that cyclic AMP was slightly elevated in the psoriatic plaque as compared with uninvolved skin of the same patient or with normal skin.

Such contradiction is in part due to the difficulties involved in measurements carried out on keratome slices, which invariably contain unknown amounts of stratum corneum and dermis. In addition, in the case of a compound like cyclic AMP, which has been shown in some tissues to change its level very rapidly after removal from the body (Kakiuchi & Rall, 1968; Ditzion, Paul & Pauk, 1970; Uzunov & Weiss, 1971; Steiner, Ferrendelli & Kipnis, 1972), the tissue must be fixed prior to removal or immediately after removal.

To overcome the difficulties of tissue selection we have applied microdissection to freeze-dried slices (Lowry, 1953) obtained from punch biopsies of skin frozen with ethyl chloride spray prior to biopsy. In this manner we were able to obtain more than 95% pure epidermis, dermis or skin append-

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ages. The microdissection of pure epidermis from a 20 μm thick frozen-sliced section yielded a tissue specimen weighing approximately 1·5 μg. Many samples have to be dissected and pooled to obtain enough tissue for assay. Therefore in conjunction with the microtechnique it was also necessary to establish a micro-assay system which would reliably measure the amount of cyclic AMP in 50–100 μg of tissue. In this communication, we describe the measurement of cyclic AMP in microdissected skin tissue using Gilman's protein binding assay method (1970) modified to fit this purpose.

MATERIALS

Sample preparation

Preliminary experiments were done on tissue obtained from the back of normal adult men by use of a keratome adjusted to a 0·2 mm setting without the use of anaesthesia, then frozen immediately in liquid nitrogen. The tissue was then cut in a cryostat at 20 μm in thickness, lyophilized at −30°C, and used without further dissection. For the actual *in vivo* cyclic AMP measurements in various components of human skin, the skin was frozen with a spray of ethyl chloride, and a punch biopsy (6·0 mm) was obtained by use of a motor driven biopsy punch, keeping the biopsy samples frozen at all times. The biopsy was then cut in a cryostat at 20 μm in thickness and vertical to the skin surface. Epidermis and dermis were dissected free-hand under a stereomicroscope after lyophilization of the cryostat sections at −30°C. Hair follicles were plucked from the scalp and beard of adult men, frozen within 2 s on a block of dry ice, lyophilized under the same conditions, and dissected into bulb and sheath portions; the latter was further dissected free from hair cortex. Tissue components were weighed on a Cahn RG electrobalance.

Reagents

^{3}H-cyclic AMP (Sp. Act. = 24·1 Ci/mmol) was used (New England Nuclear). Protein kinase (cyclic AMP binding protein) and its inhibitor prepared from beef heart were obtained (Sigma Chemical Co.). Protein kinase was obtained lyophilized, dissolved in water and kept at −60°C. Binding activity was checked in the presence of 0·1 pmol of ^{3}H-cyclic AMP and the amount of binding protein necessary to bind about 40% of the cyclic AMP was determined. Protein kinase inhibitor was used in the amount that is said by the company to increase the binding by a factor of two.

Millipore filter membranes, 25 mm discs with a pore size of 0·45 μm, were purchased from Millipore Corp.

Scintillation counting medium was prepared by dissolving 4 g of Omnifluor (New England Nuclear) and 20 ml of Biosolv BBS 3 (Beckman) in 1 litre of scintillation grade toluene.

METHODS

The original Gilman's method calls for precipitation of protein in the tissue by homogenization with trichloracetic acid (TCA) followed by extraction of the TCA by repeated washes with water-saturated ether. This procedure was not practical in our study for several reasons: (1) Micro-amounts of tissue cannot be adequately homogenized with TCA. This leads to inadequate extraction of the cyclic AMP. (2) Removal of TCA by ether is difficult on a microscale and led to some loss of the tissue extract. (3) Unpredictable results were obtained after the ether washed supernatant was lyophilized and assayed (probably due to adenine nucleotides other than cyclic AMP). Modifications designed in this work were essentially (a) the extraction of cyclic AMP in tissue with HCl, which was removed by freezing and drying, (b) the removal of interfering nucleotides by $ZnSO_4$–$Ba(OH)_2$ precipitation and (c) a micro-scale adaptation of Gilman's method.

Freeze-dried tissues weighing 30–200 μg were placed in micro test tubes (2·5 × 35 mm). Following the addition of 30 μl of 0·1N HCl, the tubes were sealed (parafilm and an overseal of adhesive tape), placed in a boiling water bath for 5 min, removed from the bath and mixed vigorously (Vortex mixer), and centrifuged at 2500 *g* for 20 min. The supernatants were transferred to fresh tubes of the same size, 20 μl of 0·1 N HCl, was added to the precipitates and the process of mixing and centrifuging repeated. The second supernatants were combined with the first, lyophilized, and then reconstituted with 30 μl of distilled water. Ten microlitres of about 0·3N $ZnSO_4$ and 10 μl of 0·3N $Ba(OH)_2$ were added to the reconstituted supernatants,* the tubes were placed in an ice bath for 10 min, and then centrifuged at 2500 *g* for 20 min.

Thirty-five microlitres of the resultant supernatants were transferred to reaction tubes (5 × 50 mm) and the cyclic AMP binding assay was carried out by adding 5 μmols of acetate buffer pH 4·0, 0·1 pmol of ^{3}H-cyclic AMP, 8 μg of protein kinase inhibitor, and enough protein kinase to bind 0·04 pmols of cyclic AMP. The total volume was kept to 50 μl. The mixture was kept at 0°C for 60–90 min, then diluted with 500 μl of chilled 20 mM potassium phosphate buffer pH 6·0, and allowed to stand for 5 min in an ice bath. At the end of this period, the mixture was passed through a millipore filter and the filter was washed with 20 ml of the same buffer. The radioactivity on the membrane was counted in 10 ml of scintillation counting medium.

Standards consisting of known amounts of non-radioactive cyclic AMP (0·03–1 pmol) were added to the reaction mixture and incubated simultaneously. The mixture also contained 35 μl of a supernatant obtained by mixing $ZnSO_4$, $Ba(OH)_2$ and H_2O in the same ratio as used in the purification of tissue extract. To correct for loss during the cyclic AMP extraction and purification procedure, we added 0·1 pmol of ^{3}H-cyclic AMP to additional tissue samples and processed them in the same way as the assay specimen. After the $ZnSO_4$–$Ba(OH)_2$ precipitation step, radioactivity in 35 μl of these supernatants was counted to calculate the recovery up to the end of the purification step. The recovery ranged between 50 and 60%.

RESULTS AND DISCUSSION

Standard Curve

In a competitive binding assay the amount of radioactive substrate should approximate to the non-radioactive. Since the expected weight of dry tissue to be examined was in the order of 100 μg we expected to be measuring approximately 0·1 pmol of cyclic AMP in the tissue samples. The original Gilman assay system uses 1 pmol of ^{3}H-cyclic AMP or ten times the range which would be useful for microdissection studies. Scaling down the amount of ^{3}H-cyclic AMP (and binding protein) used in the assay brings the concentration of cyclic AMP to below the Km for activation of protein kinase (binding protein) by cyclic AMP (0·5 pmol/50 μl of fluid). Despite this fact the results demonstrated that amounts of ^{3}H-cyclic AMP as low as 0·1 pmol can be used and give almost complete binding as shown by the linear relationship of binding of ^{3}H-cyclic AMP v. total cyclic AMP on the log plot. Linearity is maintained down to approximately 0·15 pmol total (0·1 pmol of ^{3}H-cyclic AMP plus 0·05 pmols non-radioactive cyclic AMP). Below this level there is a slight convex curve indicating less than complete binding. This is not due to the presence of $ZnSO_4$-$Ba(OH)_2$ precipitation supernatant in the reaction mixture, because we observed essentially the same standard curve in the presence and absence of this supernatant. The non-linear portion of the curve increases as the binding protein ages. For this reason a complete standard curve should be run with each set of assays.

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* The concentration of $ZnSO_4$ was periodically adjusted so that when equal quantities of $ZnSO_4$ and $Ba(OH)_2$ solution were added together, the resultant pH was 7·5 (Krishna, Weiss & Brodie, 1968; Krishna & Birnbaumer, 1970).

Equilibration of Binding Reaction

As the cyclic AMP binding reaction was carried out at a cyclic AMP concentration some ten times lower than that used in the original method, equilibration of the reaction was re-examined using

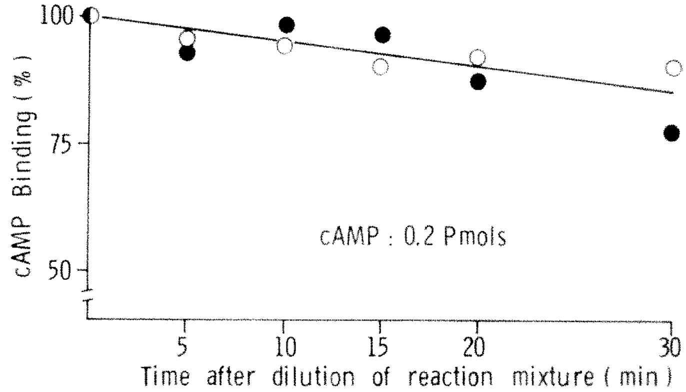

FIGURE 1. Dissociation of cyclic AMP-protein kinase complex after dilution of the reaction mixture. The reaction system is the same as described in the text except for the absence of non-radioactive cyclic AMP in the reaction mixture (and with 0·2 pmol of ^{3}H-cyclic AMP in the incubation). The reaction mixture was diluted with 500 μl of ice-cold 20 mM potassium phosphate buffer pH 6·0, and after the indicated periods in ice bath, applied to the millipore membrane followed by washing with 20 ml of the same buffer. ○ and ● show the results of each series of experiment where every point indicates an average of duplicate measurements. Nearly identical data were obtained with 0·1 pmol of ^{3}H-cyclic AMP.

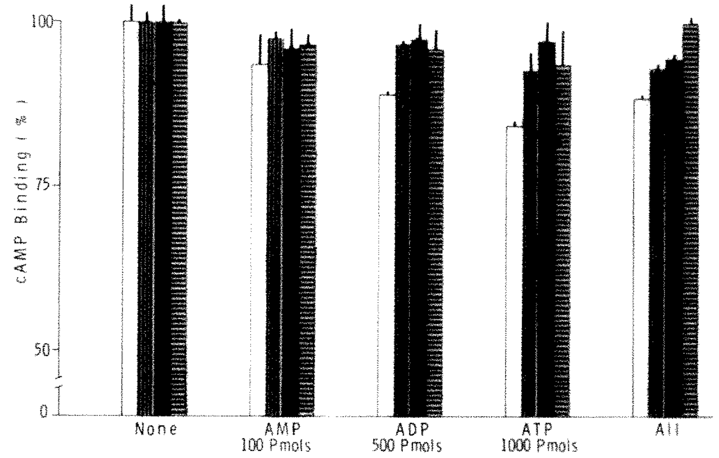

FIGURE 2. Effect of other nucleotides on cyclic AMP binding. Binding was measured with 0·1 pmol (▤) or 0·2 pmol (□ , ▥ , ■) of ^{3}H-cyclic AMP in the reaction system, with or without various nucleotides which were added directly to the reaction mixture (▤, ▥), or boiled for 5 min in 0·1 N HCl, lyophilized, redissolved in water, then added to the reaction mixture before (▥) or after (■) Ba(OH)$_2$-ZnSO$_4$ precipitation treatment. Concentration of nucleotides was adjusted to avoid the dilution by this treatment. Data are expressed as a percentage of the binding without any other nucleotide in the reaction mixture. The average of duplicate measurements and the extent of variations are shown.

various incubation periods. The binding proceeded as fast as in the original system and reached equilibrium well before 30 min, and stayed the same for at least 2 h. Incubation in all further experiments was therefore carried out for 60–90 min.

Dissociation of the cyclic AMP-protein kinase complex after dilution of the reaction mixture

Although the binding complex is quite stable (first order dissociation with half-time of 7 h) in the original system, it showed a more rapid dissociation in this system after dilution of the reaction mixture with 500 μl of 20 mM potassium phosphate buffer pH 6·0, and 10–15% dissociation was observed after 30 min. Slightly more dissociation was noted when the cyclic AMP concentration was raised from 0·1 pmol to 0·2 pmol (Fig. 1). It is important, therefore, to finish the washing procedure as soon as possible after dilution of the reaction mixture, or to correct for this dissociation by appropriate placement of standards.

Effects of other adenine nucleotides on binding

The effects of AMP, ADP and ATP on cyclic AMP binding were examined in the range of their probable *in vivo* concentrations (Kondo & Adachi, 1971; Decker, 1971; Hammar, 1973). All of these compounds when added directly to the binding assay mixture inhibited the binding to a certain degree (Fig. 2). Boiling of these compounds in 0·1N HCl for 5 min reduced this inhibitory effect. Further treatment with $ZnSO_4$ and $Ba(OH)_2$ also reduced the inhibitory effect of these nucleotides, and showed the necessity of these steps in the assay.

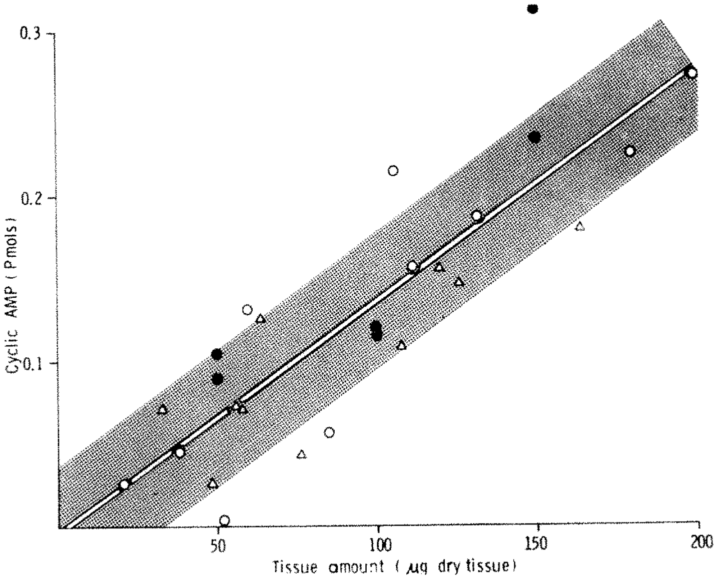

FIGURE 3. Cyclic AMP content in various amounts of tissue. Tissues were obtained from the back of two white adult males (a and b) by keratome (setting, 0·2 mm), quickly frozen and thin sectioned in cryostat (20 μm), then lyophilized and analysed. ○, Tissue from a; △, Tissue from b; ●, indicates the value obtained with tissue from a, 1 mg dry weight of which was boiled, homogenized with 500 μl of 0·1N HCl, and aliquots containing corresponding amounts of tissue were analysed. The following computer statistical analysis of the data was carried out by courtesy of Dr T. Johnson of the Department of Radiology, VA Hospital, Miami: Y = 0·0013; X=0·0048; R = 0·9; s.d. of sample from regression = 0·04; s.e. of the slope = 0·0002; $n = 26$. Shaded zone indicates 95% confidence limits of the slope.

These results also exclude the possibility of a significant amount of non-enzymatic cyclic AMP formation from endogenous ATP by $ZnSO_4$-$Ba(OH)_2$ during the purification step. (In Fig. 2, ATP was added in 5000 times larger amounts than cyclic AMP. If a significant amount of cyclic AMP formation took place during $ZnSO_4$-$Ba(OH)_2$ treatment this newly formed cyclic AMP would inhibit the binding of ^3H-cyclic AMP.)

Assay of cyclic AMP in pooled freeze-dried sections of skin

The sections were removed with a keratome (each slice contained some stratum corneum and some dermis also).

Various amounts of freeze-dried tissue from two keratome slices were analysed for cyclic AMP content. In addition, larger pieces of freeze-dried skin were boiled and homogenized with 0·1N HCl and aliquots corresponding to the same range as the weights of freeze-dried tissue were also analysed. This allowed a check on the completeness of extraction by 0·1N HCl. All the data points demonstrate an acceptable relationship for cyclic AMP content found versus weight of tissue utilised. This validates the technique for use in definitive experiments measuring cyclic AMP in separated components of skin.

Recovery

Two different amounts of non-radioactive cyclic AMP (0·063 & 0·125 pmols) were added to 100 μg

TABLE 1. Recovery test

	Cyclic AMP detected (pmols)	Mean ± s.e.	2 − 1 of Mean
Experiment 1			
(1) Tissue 100 μg	0·260		
dry weight	0·204		
each	0·148	0·179 ± 0·024	—
	0·120		
	0·163		
(2) Tissue 100 μg	0·209		
plus cAMP	0·274		
0·063 pmols	0·254	0·253 ± 0·015	0·074
each	0·295		
	0·234		
Experiment II			
(1) Tissue 100 μg	0·225		
dry weight	0·142		
each	0·161	0·170 ± 0·014	—
	0·175		
	0·145		
(2) Tissue 100 μg	0·285		
plus cAMP	0·297		
0·125 pmols	0·314	0·298 ± 0·009	0·128
each	0·322		
	0·272		

Tissue utilized in these experiments was from the same piece of skin obtained with keratome.

of tissue and their recoveries were examined using the same extraction and binding assay procedure as for tissue. Recovery of exogenously added cyclic AMP was nearly 100% as shown in Table 1.

Cyclic AMP content in the various components of skin

The results are summarized in Table 2. The dermis showed a cyclic AMP content of 1·13 pmol/mg dry tissue. This is about a half of the epidermal content, and is probably due to the relative lack of cells in the dermis. Single hair follicles of the beard showed a larger cyclic AMP content than that of scalp hair follicles. But, when the size of both hair follicles was taken into consideration (the beard hair follicle is 2 to 3 times larger than the scalp hair follicle), the content in the beard follicle may be lower. This is due to a lower cyclic AMP content in the sheath portion of beard follicle as shown in the table on the basis of mg dry tissue weight.

TABLE 2. Cyclic AMP content in the skin components

	Cyclic AMP content	
Tissue	pmols/mg dry wt. Mean ± s.e. (# assay)	pmols/single unit Mean ± s.e. (# assay)
Epidermis (I)	2·76 ± 0·75 (4)	
(II)	1·67 ± 0·26 (4)	
Average	2·22	
Dermis (I)	1·25 ± 0·19 (4)	
(II)	1·01 ± 0·26 (3)	
Average	1·13	
Hair follicle		
Scalp sheath	2·72 ± 0·16 (5)	0·17 ± 0·03 (9)
bulb	3·50 ± 0·90 (2)	0·06 ± 0·02 (4)
whole		0·23
Beard sheath	1·83 ± 0·18 (11)	0·22 ± 0·02 (14)
bulb	3·54 ± 0·41 (4)	0·09 ± 0·01 (4)
whole		0.31

Data on epidermis and dermis are those of uninvolved skin from two white adult males with psoriasis. Data on hair follicles were obtained from two Japanese adult males. Values for whole follicles were the sum of the mean values of the sheath portion and bulb portion. The following numbers of hair follicles were required to prepare tissue for a single assay. Scalp hair sheath, 1–2; scalp hair bulb, 3–4; beard sheath, 0·5–1; beard bulb, 1–3.

Although variations in the results presented in Fig. 3 and Table 1 are due to some extent to poor homogeneity of the tissue (i.e. the tissues used in these experiments were obtained by use of a keratome, and, therefore, contained variable amounts of contaminating dermis and stratum corneum), additional variations due to microscale tissue extraction cannot be avoided. At least triplicate assays are required to obtain reproducible results. A 6 mm punch biopsy specimen from normal human skin will provide ample epidermal tissue for 4–5 cyclic AMP assays (60–70 μg dry weight for each assay) plus a sample to assess recovery, and enough for other analyses (a micro protein and DNA determination, etc.). Thus, this procedure for micro cyclic AMP measurements may be applied successfully for the biochemical analysis of human pathological skin.

ACKNOWLEDGMENT

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Cyclic AMP in skin: effects of acute ischaemia

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SUMMARY

The cyclic adenosine 3′,5′-monophosphate (cyclic AMP) content of pig skin was measured several seconds to minutes after removal of the skin from the body. It increased very rapidly, reached a maximum by 2 min after removal (4 times higher than the initial level), then decreased very slowly.

Propranolol injected into the animal before or added after the removal of the skin did not suppress this phenomenon. The practical significance of this finding (increase of cyclic AMP level in skin after ischaemia) is obvious—in order to measure cyclic AMP level *in vivo*, the sample must be frozen immediately to avoid an 'artificial' increase in cyclic AMP.

It has been reported that the level of brain cyclic AMP (adenosine 3′,5′-monophosphate) increases rapidly after decapitation, reaches its maximum within 1–2 min, and then rapidly decreases to the initial level within 5 min (Kakiuchi & Rall, 1968; Ditzion, Paul & Pauk, 1970; Steiner, Ferrendelli & Kipnis, 1972). This phenomenon does not occur in all mammalian tissues. As reported by Ebadi, Weiss & Costa (1971), of six different tissues studied only cerebellum and heart showed an increase in cyclic AMP content due to ischaemia, while liver, kidney and pineal showed a gradual or rapid decrease in cyclic AMP levels in a 5 min period post mortem. Even in the brain this phenomenon is much less remarkable in the cerebrum and brain stem than in the cerebellum (Uzunov & Weiss, 1971), suggesting a very restricted tissue specificity.

In spite of many studies, the mechanism which causes this phenomenon is not known and it is simply considered a result of ischaemia. Some phenothiazine tranquillizers are known to suppress it in brain and their effect parallels their sedative activity (Uzunov & Weiss, 1971).

On the basis of the reults reported here, skin must be added to the list of tissues which respond to ischaemia by an acute rise in cyclic AMP.

MATERIALS AND METHODS

Young domestic pigs weighing about 20 kg were obtained from a local farm. The dorsal skin was shaved and wiped gently with water, and a sheet of skin consisting of epidermis and upper dermis

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was obtained without anaesthesia by the use of a disposable keratome or a Castroviejo keratome set at 0·4 mm. The skin thus obtained was quickly cut into several pieces and maintained in a laboratory atmosphere for a carefully determined number of seconds or minutes after removal before being frozen between solid dry ice pieces. Initial or zero second specimens were obtained after freezing the skin with an ethyl chloride spray. Frozen specimens were subsequently lyophilized at −30°C and kept in a vacuum at the same temperature until assayed. Tissue pieces of 5–7 mg dry weight were boiled for 5 min and then homogenized in a glass homogenizer with 1 ml of 0·1N HCl. Two hundred microlitres of the homogenates were centrifuged at 2500 *g* for 20 min. The supernatants were lyophilized and reconstituted with 100 μl of H_2O followed by mixing with 100 μl each of nearly 0·3N solutions of $ZnSO_4$ and $Ba(OH)_2$. The concentration of the $ZnSO_4$ solution was periodically adjusted so that the pH was 7·5 when equal quantities of these solutions were mixed together (Krishna, Weiss & Brodie, 1968).

The mixtures were centrifuged at 2500 *g* for 20 min, and 60 μl of the resultant supernatants were analysed for cyclic AMP content in duplicate. Standard cyclic AMP specimens were also assayed at the same time with 60 μl of the supernatant prepared from mixing H_2O, $Ba(OH)_2$ and $ZnSO_4$ in the same ratio. The recovery of cyclic AMP up to this step was calculated by the addition of radioactive cyclic AMP at the initial step. Taking ^3H-cyclic AMP in 200 μl tissue homogenate as 100%, 16 ± 0·5% of radioactive cyclic AMP was constantly recovered in 60 μl of this supernatant (about 70% in total supernatant). No significant degradation of cyclic AMP during the boiling with 0·1N HCl was observed.

Cyclic AMP content was measured in a total reaction mixture of 100 μl by the protein kinase binding method of Gilman (1970) with 1 or 2 pmols of ^3H-cyclic AMP (New England Nuclear, S.A. = 22·1 Ci/mmol) and protein kinase and its inhibitor protein (Sigma Chemical Co.). The concentration of acetate buffer pH 4·0 in the reaction mixture was increased to 100 mM from the original 50 mM. The radioactivity was counted in 10 ml of counting medium prepared with 4 *g* of Omnifluor (New England Nuclear), 20 ml of Bio-solv BBS 3 (Beckman) and 1 litre of scintillation grade toluene.

The tissue extract with or without addition of exogenous cyclic AMP was digested by the specific phosphodiesterase, following which no cyclic AMP was detected by the above procedures.

RESULTS

The cyclic AMP content of pig skin increased very rapidly after removal of the skin from the body. Within 10–20 s the level had doubled. Two minutes later, when the cyclic AMP content was about 4 times as high as that of the initial value, it levelled off until 5 min after removal (Fig. 1). A very gradual decrease of cyclic AMP content was observed in the period from 5–20 min, but by 20 min the level was still significantly higher than the initial level (data not shown).

It has been shown in brain (Lowry *et al.*, 1964) and also skin (Kondo & Adachi, 1971) that the levels of metabolic intermediates and related nucleotides of the glycolytic pathway change very rapidly after decapitation or removal of skin from the body. It is difficult to understand how these changes might be a direct cause of the cyclic AMP increase because ATP which is a substrate of cyclic AMP formation was decreased and not increased by ischaemia. It has previously been shown in rat and human skin that β-adrenergic agents strongly stimulate cyclic AMP formation *in vitro* (Powell, Duell & Voorhees, 1971; Haia *et al.*, 1972). This effect can be blocked by β-adrenergic blocking agents (Voorhees *et al.*, 1972). This is also true in pig skin (Table 1).

Since propranolol can block the rise in cyclic AMP caused by catecholamines, the effect of propranolol on the ischeamic rise of cyclic AMP was studied. A systemic or local increase of catecholamines induced by the stress of sampling might have played a role in this cyclic AMP increase.

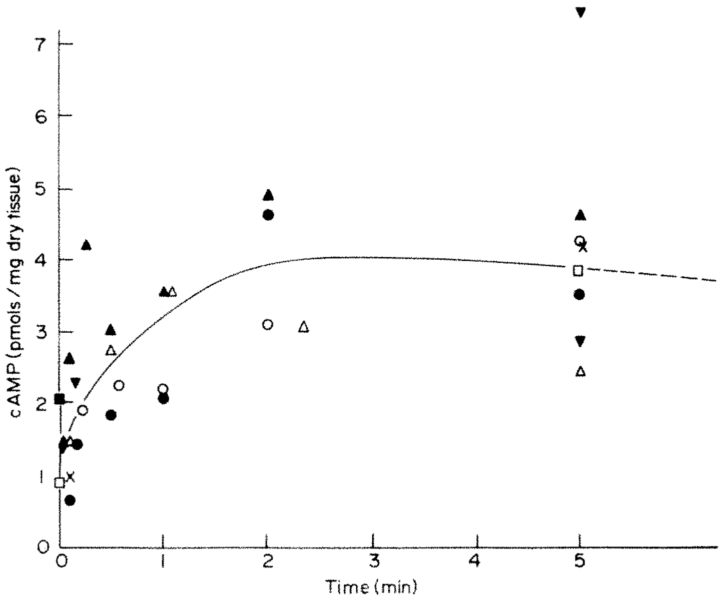

FIGURE 1. Change in cAMP level in pig skin after removal from the body. The same symbol indicates a series of experiments. Data were obtained with specimens from six animals. All specimens were obtained by use of a disposable keratome.

TABLE 1. *In vitro* effects of adrenaline, theophylline, and propranolol on cAMP accumulation in pig skin

Incubation condition	cAMP (pmols/mg dry tissue)		
	Exp. 1 (10 min)	Incubation	Exp. 2 (5 min)
Hank's balanced salt sol.	3·47		3·90
+Theophylline (2 mM)	—		4·01
+Adrenaline (5×10^{-5} M)	8·83		10·20
+Theophylline (2 mM) and Adrenaline (5×10^{-5} M)	20·78		15.80
+Propranol (5×10^{-4} M)	1·65		3·15
+Adrenaline (5×10^{-5} M) and Propranolol (5×10^{-4} M)	2·69		—
+Theophylline (2 mM), Adrenaline (5×10^{-5} M) and Propranolol (5×10^{-4} M)	3·69		5·51

The skins obtained by a Castroviejo keratome were kept in Hank's balanced salt solution at 4°C and used about 40 min after removal without pre-incubation. Incubation was carried out at 37°C for the designated periods followed by quick freezing of the tissues between pieces of dry ice. Frozen-fixed tissues were analysed for cAMP content as described in the text.

Fig. 2a shows the results with specimens obtained 20 min after the intravenous injection of propranolol HCl in amounts of 0·1 mg or 1 mg/kg body weight. Fig. 2b shows the results with specimens removed 5 min after the local injection of 1 mg of propranolol HCl. In all these experiments, the cyclic AMP level after removal of the skin showed an acute elevation with the same pattern as the control skin did (Fig. 1). Propranolol at this dosage failed to suppress the increase of cyclic AMP after removal.

In addition tissue was obtained from non-injected animals. A portion was immediately frozen and the remaining part of the specimen was quickly divided into pieces which were exposed for 5 min to the conditions shown in Table 2. Again propranolol failed to suppress the increase in cyclic AMP. The propranolol concentration used (5×10^{-4} M) was one which showed almost complete blocking

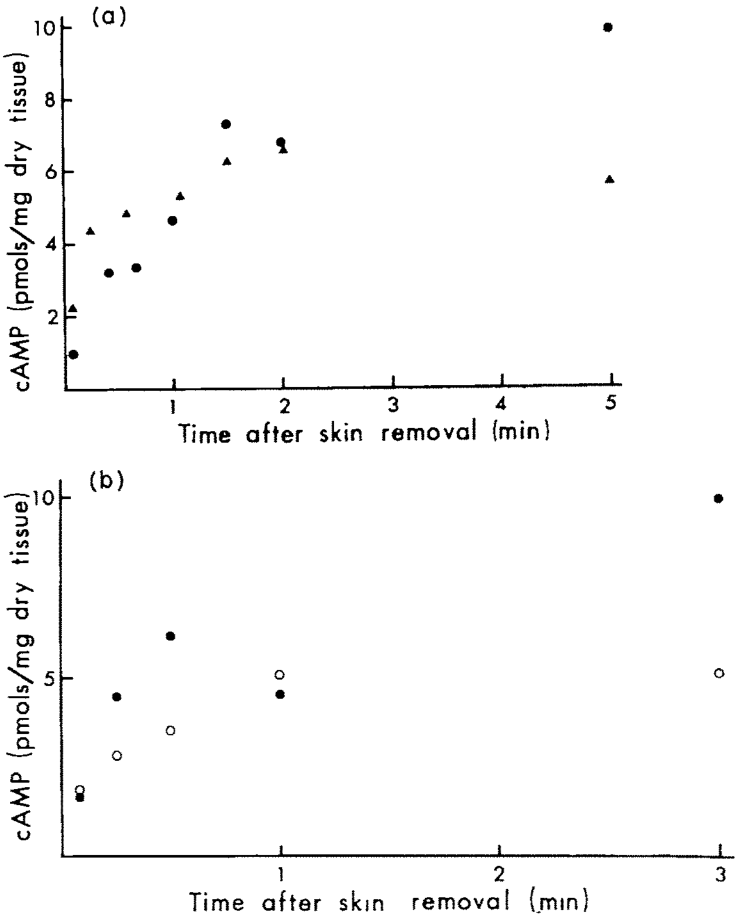

FIGURE 2. Change in cAMP level in pig skin removed after the injection of propranolol HCl into animals.

(a) intravenous injection 20 min before sampling (●, 0·1 mg/kg; ▲, 1 mg/kg of the drug were injected through the ear vein). Data are on different animals.

(b) Local injection 5 min before sampling (●, 1 mg propranolol HCl/ml; ○, 0·9% NaCl 1 ml quantity were intradermally injected diffusely in approximately a 2·5 × 5 cm area. Propranolol was injected into the same animal after sampling the control site injected with 0·9% NaCl).

All specimens were obtained with a 0·4 mm setting of a Castroviejo keratome.

TABLE 2. Change of cyclic AMP content in pig skin under various conditions for 5 min after removal from the body

Condition	Cyclic AMP content (pmols/mg dry tissue) in the experiments									
	1	2	3	4	5	6	7	8	9	Average (s.e.)
Frozen 5 s after removal	Not detected	0·97	2·31	—	—	—	—	—	—	< 1·64
In room atmosphere	4·31	9·13	7·44	—	—	—	—	—	—	6·96
In Hank's balanced salt solution	6·14	4·14	4·10	—	—	—	—	—	—	4·79
In 0·9% NaCl	3·86	4·33	2·87	2·99	1·26	2·43	2·32	2·47	2·50	2·78 (±0·23)
In 0·9% NaCl + propranolol (5×10^{-4} M)	1·64	3·21	3·19	4·23	4·89	5·86	4·35	2·31	2·34	3·56 (±0·46)

of the effect of 5×10^{-5} M adrenaline *in vitro* as shown in Table 1. The cyclic AMP level increase was less remarkable in the specimen placed in saline media than that kept in room atmosphere. The reason is unknown.

DISCUSSION

It is obvious from this study that the cyclic AMP content of the skin increases rapidly after removal from the body. The rise is only slightly slower than that observed in brain tissue. However, a remarkable difference between the skin and the brain is shown by the rate of the cyclic AMP decrease after it reaches its maximum level. In the brain, the level reaches its maximum within 2 min then goes down quickly to return to the initial level 5 min after removal (Ditzion *et al.*, 1970; Steiner *et al.*, 1972). On the other hand in the skin the cyclic AMP level reaches its maximum about 2 min later, stays at the same level for 3 more min and then starts to decrease very slowly (Fig. 1).

It is not clear from this experiment whether dermis or epidermis is most responsible for this phenomenon, because the specimen was 0·4 mm in thickness and contained about equal amounts of the two components. It was technically very difficult to obtain thinner specimens because we took the specimens from animals without anaesthesia. Yet, the data presented do show the rapid increase of cyclic AMP after removal, and the ineffectiveness of the β-adrenergic blocking agent propranolol in suppressing this phenomenon. This suggests that the rapid release of a β-adrenergic agent is unlikely to be a causative factor. In conclusion it should be pointed out that skin samples for the measurement of cyclic AMP content have to be very carefully handled. As little as a 10 s delay after removal of the skin nearly doubles the cyclic AMP content. In addition local infiltration anaesthesia could cause a relatively ischaemic situation *in vivo* by compressing the local circulation even if the anaesthetic itself did not pharmacologically activate cyclic AMP metabolism.

ACKNOWLEDGMENT

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Further studies on adenyl cyclase in psoriasis

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SUMMARY

Slices of human skin obtained with a keratome were pre-incubated with [^{3}H]adenine to label the ATP pool from which cyclic AMP was subsequently formed. The accumulation of radioactive cyclic AMP was measured as an index of adenyl cyclase activity. The data showed that both the ability to incorporate [^{3}H] into ATP and adenyl cyclase activity were significantly lower in psoriatic plaques than in uninvolved skin of the psoriatic patients, or in normal skin of control subjects. The response of adenyl cyclase to the stimulation of 3·3 μM adrenaline was less than five fold in psoriatic plaques as compared to twelve to thirty-two fold in the uninvolved skin. The response to the stimulation of prostaglandin E_2 (5 μg/ml) showed no significant difference between the plaque and normal skin. The adenyl cyclase activity in uninvolved skin of psoriatic patients appeared normal. Propranolol (10 μM) blocked the stimulatory effect of adrenaline but not that of PGE_2 in normal skin. These results suggest that the adenyl cyclase system of the skin has different regulatory sites for adrenaline and PGE_2, and that the enzyme is defective in the epidermis of the psoriatic plaque, especially at the adrenaline regulatory site.

The possibility that adenosine 3′,5′-cyclic monophosphate (cAMP) may mediate the regulation of epidermal proliferation has prompted a number of recent investigations. This possibility was suggested by Iverson (1969), based on studies of Bullough & Laurence (1964) on the role of a postulated complex of chalone and adrenaline in regulating mitosis of mouse ear epidermis. Voorhees & Duell (1971) subsequently proposed the hypothesis that there may be a defect in the 'adenyl cyclase-cAMP cascade' in psoriasis. These investigators have published a number of papers to support the hypothesis (Voorhees *et al.*, 1972, 1973a, b). A recent article by Härkönen, Hopsu-Havu & Raij (1974) indicated lowered activities of adenyl cyclase and of cyclic nucleotide phosphodiesterase in psoriasis, but the cAMP level was, however, slightly elevated.

In our previous studies (Hsia *et al.*, 1972; Hsia, Wright & Halprin, 1973; Wright *et al.*, 1973), we demonstrated adenyl cyclase activity in slices of human skin obtained with a keratome, and in cell-free preparations of the skin slices. In these studies psoriatic skin was found to have lower adenyl cyclase activity than adjacent uninvolved skin of the patients, or skin from control subjects who did not have psoriasis or other inflammatory skin disease. Furthermore, we found that the enzyme in psoriatic plaques was less responsive to the stimulation of adrenaline and NaF. In a continuation of our studies, we have improved our procedure, and obtained more convincing evidence that adenyl cyclase in the psoriatic plaque is abnormal. In addition, we have studied the effect of prostaglandin E_2 (PGE_2) on adenyl cyclase in normal and psoriatic skin. These findings are reported in this paper.

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A preliminary report on this study was given at the Second International Conference on Cyclic AMP at Vancouver, Canada, 8–11 July 1974.

MATERIALS AND METHODS

Skin Specimens

Skin specimens were obtained from psoriatic patients and control subjects who had no clinical signs of psoriasis or other inflammatory skin disease, using a keratome with the blade set at 0·2 mm, without anaesthesia. The excision in normal skin or uninvolved skin of the patients was immediately below the basal cell layer so that the specimen consisted mostly of epidermis. As the psoriatic epidermis is thicker, some of the lowermost cells in the epidermal projections were not included in the specimen. The specimens from psoriatic plaques were taken in such a way so as to include some adjacent uninvolved skin for comparative examination. After excision, the specimens were wrapped in cloth wet with saline and immediately prepared for incubation as described below.

Incubation with [^{3}H]adenine

The proceedure of Kuo & De Renzo (1969) for the study of adenyl cyclase in adipose cells was adapted. An exactly weighed sample of the 0·2 mm specimen (10–25 mg) was pre-incubated at 37°C for 60 min with 5 μCi of [^{3}H]adenine (New England Nuclear, specific activity 8·4 μCi/mmole) in 2 ml of Krebs-Ringer bicarbonate buffer containing 5·5 mM glucose, gassed to pH 7·4 under O_2:CO_2, 95:5, to allow labelling of the ATP pool. The tissue was removed from the incubation medium, rinsed with saline, and incubated for 30 min in a fresh medium of 2 ml of Krebs–Ringer bicarbonate buffer, pH 7·4, containing 10 mM theophylline with or without other additions as specified. The incubation was terminated by the removal of the tissue from the medium and rinsing it with saline. The tissue was then boiled for 5 min in 1 ml of Tris buffer, pH 7·2, containing 10 mM theophylline and 0·625 μmoles of cAMP. The latter served as internal standard for measuring the recovery of cAMP. After homogenization, the mixture was centrifuged at 650 *g* for 15 min. The supernatant fluid (0.5 ml) was chromatographed on a column of Dowex AG 50W–X4, according to the procedure of Krishna, Weiss & Brodie (1968). The fraction containing cAMP was treated with $ZnSO_4$ and $Ba(OH)_2$ to precipitate traces of ATP and ADP. An aliquot was used to determine the recovery of cAMP by measuring absorbance at 259 nm. Aliquots from the ATP fraction were assayed for ^{3}H in ATP.

RESULTS

Incorporation of [^{3}H]adenine into ATP

After incubation of [^{3}H]adenine with the 0·2 mm specimens of psoriatic plaques and uninvolved skin of the patients, the nucleotides in the supernatant of the homogenates were separated on the Dowex column. The ^{3}H in the ATP fraction obtained from each specimen was assayed. The results are shown in Table 1. The mean values of ^{3}H in the ATP fraction from psoriatic plaques was $5·24 \times 10^4$ dpm/mg skin, while that of uninvolved skin was 1.36×10^5 dpm/mg skin. The difference was statistically significant ($P<0·001$, by the matched pair *t* test). The mean value of results from skin of six control subjects was $1·05 \times 10^5$ dpm/mg skin (not shown in Table 1), and was not significantly different from the mean value of the uninvolved skin of patients. Thus, the ability to utilize

TABLE I. Incorporation of [^{3}H]adenine into ATP by skin specimens of psoriatic patients

Patient	^{3}H in ATP, dpm/mg skin Psoriatic plaque	 Uninvolved skin
	× 10^4	× 10^4
1	7·31	18·3
2	2·18	12·0
3	5·85	13·0
4	7·43	14·9
5	3·44	9·6
mean ± SEM	5·24 ± 2·34	13·6 ± 3·26

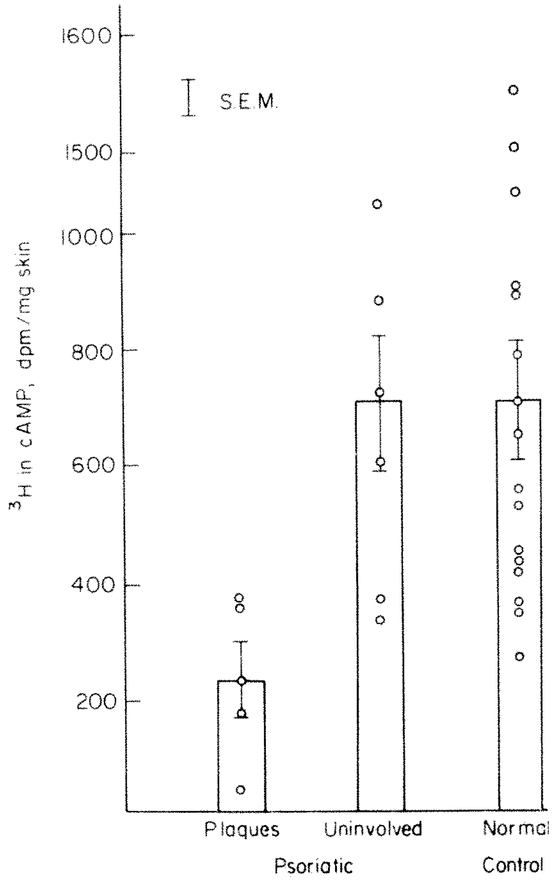

FIGURE 1. Formation of [^{3}H]cAMP by specimens of psoriatic plaques, uninvolved skin of psoriatic patients, and normal skin of control subjects. Incubation and assay procedures are described in the text. The bars represent mean ± SEM. The difference between the mean values obtained from psoriatic plaques and uninvolved skin was significant ($P = 0·005$).

[^{3}H]adenine in the formation of ATP was lower in psoriatic plaques than in uninvolved skin, which had an ability similar to normal skin.

Formation of [^{3}H]*cAMP and effect of adrenaline*

The accumulation of [^{3}H]cAMP by skin specimens from five psoriatic patients and sixteen control subjects is shown in Fig. 1. Values of mean ± SEM of results from psoriatic plaques, uninvolved skin of patients, and normal skin from control subjects were respectively: 233±62, 718±122 and 714±99 dpm/mg skin. The difference between the means from psoriatic plaques and uninvolved skin was highly significant ($P = 0{\cdot}005$, by the matched pair t test). On the other hand, there was no significant difference between the means from uninvolved skin of psoriatic patients and skin of control subjects.

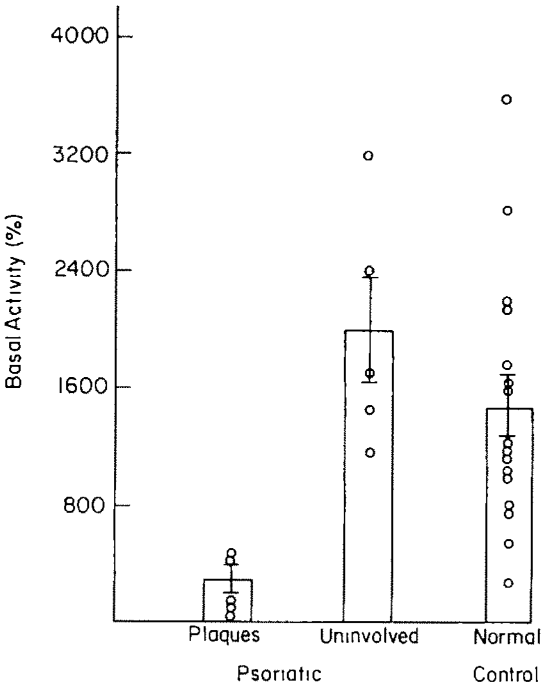

FIGURE 2. Effect of adrenaline on the accumulation of [^{3}H]cAMP. The concentration of adrenaline used was 3·3 μM. Data are expressed in percentages of basal values obtained without adrenaline. The bars represent mean ± SEM. The difference between the mean values from psoriatic plaques and uninvolved skin was significant ($P = 0{\cdot}013$).

With 3·3 μM adrenaline added to the incubation medium, the difference in the accumulation of [^{3}H]cAMP by psoriatic plaques and uninvolved skin of the patients became greater. The values of mean ± SEM were 613±288, 12990±2077 dpm/mg skin, respectively, while the values from control subjects were 10941±2864 dpm/mg skin. Fig. 2 compares these results as percentages of basal values which were obtained without adrenaline (data in Fig. 1). The response to adrenaline by psoriatic plaques (252±81%) was significantly lower than that by uninvolved skin (1996±375%) ($P = 0{\cdot}013$ by the matched pair t test). There was no significant difference between the uninvolved skin and skin of control subjects.

The response to adrenaline by psoriatic plaques and uninvolved skin of the patients was compared by another criterion, i.e. the percent conversion of [^{3}H]ATP into [^{3}H]cAMP, calculated by dividing the ^{3}H in cAMP by the sum of ^{3}H in cAMP and ATP according to the formula:

$$\frac{[^3\mathrm{H}]\mathrm{cAMP,\ dpm/mg\ skin}}{[^3\mathrm{H}]\mathrm{cAMP}+[^3\mathrm{H}]\mathrm{ATP,\ dpm/mg\ skin}}\times 100\%.$$

The data in Fig. 3 show that the percentage conversion of ATP into cAMP was lower in psoriatic plaques than in uninvolved skin, with or without 3·3 μM adrenaline in the incubation medium. The mean ± SEM of these percentages for plaques and uninvolved skin in the absence of adrenaline were 0·48 ± 0.38 and 0·76 ± 0·57%, respectively ($P = 0{\cdot}05$); and with the addition of 3·3 μM adrenaline were 1·24 ± 0·99 and 8·53 ± 1·50%, respectively ($P = 0{\cdot}002$). Thus, in addition to a decreased ability to incorporate [^{3}H]adenine into ATP, psoriatic plaques converted smaller percentages of the [^{3}H]ATP into [^{3}H]cAMP than did uninvolved skin. This difference was particularly magnified in the presence of adrenaline.

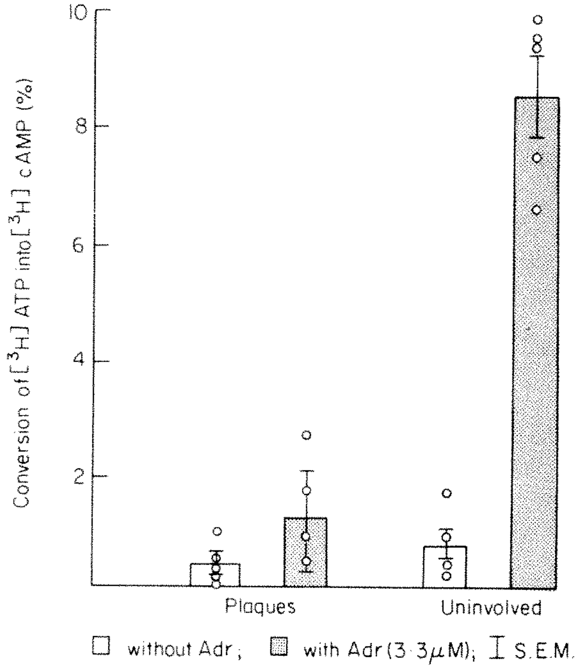

FIGURE 3. Percentage conversion of [^{3}H]ATP into [^{3}H]cAMP by psoriatic plaques and uninvolved skin of patients. Percentage conversion was calculated by

$$\frac{[^3\mathrm{H}]\mathrm{cAMP,\ dpm/mg\ skin}}{[^3\mathrm{H}]\mathrm{cAMP}+[^3\mathrm{H}]\mathrm{ATP,\ dpm/mg\ skin}}\times 100\%.$$

The bars represent mean ± SEM.

Effect of PGE$_2$ and propranolol

Fig. 4 shows that PGE$_2$ stimulated the accumulation of [^{3}H]cAMP in the skin of a normal subject. Propranolol blocked the stimulatory effect of adrenaline, but not that of PGE$_2$, while propranolol by itself had no effect. In a study with skin from a psoriatic patient, the responses to the stimulation by PGE$_2$ (14·7 μM) by the psoriatic plaque and uninvolved skin were 980% and 1160%, respectively.

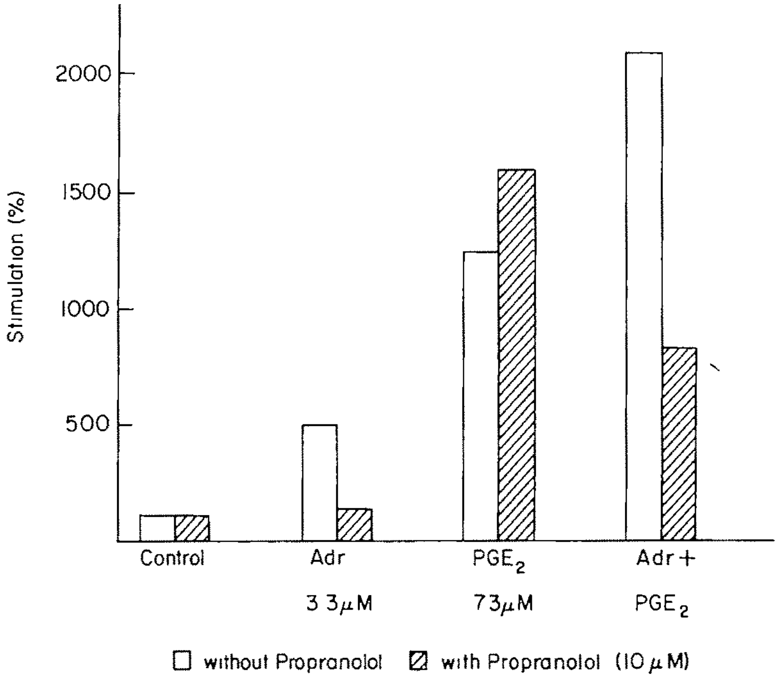

FIGURE 4. Effects of adrenaline, PGE_2 and propranolol on the accumulation of [^{3}H]cAMP in skin of a normal subject. The concentrations of adrenaline, PGE_2 and propranolol used were 3·3 μM, 7·3 μM and 10 μM, respectively. Propranolol blocked the effect of adrenaline, but not that of PGE_2.

DISCUSSION

Results from the present study confirm our previous observations that the adenyl cyclase system is defective in the epidermis of the psoriatic plaque (Hsia *et al.*, 1972, 1973; Wright *et al.*, 1973). Table 1 shows that psoriatic plaques incorporated less [^{3}H]adenine into ATP than did normal skin or uninvolved skin of the patients. Since the ATP concentration is higher in psoriatic skin than in normal skin (Hammar, 1973), our finding does not reflect inability to form ATP in psoriasis, but rather an abnormality in the 'salvage pathway' for the incorporation of adenine into ATP. This pathway begins with the condensation of adenine with α-5-phosphoribosyl-1-pyrophosphate (PRPP) leading to the formation of 5′-AMP by phosphoribosyl transferase. 5′-AMP is phosphorylated by pre-existing ATP to form ADP in a reversible reaction mediated by adenylate kinase. ADP is then phosphorylated to form ATP. The site of the enzymic defect in this pathway in psoriasis is yet to be determined. It is also possible that a greater amount of endogenous adenine was present in psoriatic plaques so as to dilute the labelled substrate. This possibility needs to be examined in further studies.

The data in Fig. 1 further show that the conversion of the newly synthesized [^{3}H]ATP into [^{3}H]cAMP was also lower in psoriatic skin than in normal skin or uninvolved skin of the patients. The defect is most likely in adenyl cyclase. Our previous study using broken cell preparations of human epidermis for the incubation with [α-^{32}P]ATP (Wright *et al.*, 1973), as well as a recent study by Härkönen *et al.* (1974), also indicated lower adenyl cyclase activity in psoriatic plaques. The abnormality in adenyl cyclase was particularly prominent in the presence of adrenaline. With

3·3 μM adrenaline in the incubation medium, adenyl cyclase activity in uninvolved skin was stimulated twelve to thirty-two fold, while in psoriatic plaques, not more than five fold (Fig. 2). These data show more clearly the defect in adenyl cyclase than our previous results (Hsia *et al.*, 1972, Wright *et al.*, 1973). The improvement in methodology was attributed to the addition of 5·5 mM glucose to the pre-incubation medium. In a control study, a dose-dependent effect of glucose in the pre-incubation medium on the response to adrenaline by normal skin was demonstrated (Hsia *et al.*, 1974). Maximum response to adrenaline was obtained with 5·5 mM glucose, which was used in the present study. As the available [^{3}H]ATP for the synthesis of [^{3}H]cAMP was less in psoriatic plaques, it was necessary to assess the percentage of conversion of [^{3}H]ATP into [^{3}H]cAMP and compare values from psoriatic plaque and uninvolved skin. The comparison is made in Fig. 3. The percentage of conversion of ATP into cAMP by the plaques was again significantly lower than by uninvolved skin. This difference was magnified in the presence of 3·3 μM adrenaline.

From these data, the defect in the adenyl cyclase system in psoriasis appears clear, the defect in response to adrenaline being a prominent feature. It is noteworthy that the response to adrenaline was calculated as a percentage of the basal value which was obtained from the same specimen in the absence of adrenaline, so that individual variations in sample thickness, number of viable cells, amounts of dermal contribution, etc., are minimized.

The results in Fig. 4 show that adenyl cyclase in the skin responds to the stimulation of PGE_2, and that propranolol (10 μM) blocked the stimulatory effect of adrenaline, but not that of PGE_2. In psoriasis, in spite of the defect in response to adrenaline, the response to PGE_2 was essentially normal. Thus, adenyl cyclase in human skin has different regulatory sites responsive to adrenaline and PGE_2. The defect in the psoriatic plaque involves particularly the adrenaline regulatory site.

There is accumulating evidence that cAMP may mediate the regulation of cell proliferation, thus a defect in the adenyl cyclase system suggests a causal relationship to the increased cellular turnover in psoriasis. However, the possibility exists that our observed abnormalities in adenyl cyclase activity may be the result rather than the cause of the disease.

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Dermal-epidermal deposition of complement components and properdin in systemic lupus erythematosus

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SUMMARY

Skin lesions and clinically normal skin of thirteen patients with systemic lupus erythematosus (SLE) were examined, by the use of immunofluorescent techniques, to determine the presence of C1q, C3, C3 proactivator (C3PA), properdin, immunoglobulins (IgG, IgA, and IgM), and fibrin. IgM deposition was present in all thirteen skin lesions and in all eleven normal areas tested, whereas IgG deposition occurred in eleven of the lesions but in only six normal areas. IgA was the least frequently encountered immunoglobulin.

C1q deposition occurred in all thirteen skin lesions, and C3 deposition was present in twelve. Seven of nine and eight of eleven clinically normal areas demonstrated C1q and C3 deposition, respectively. Although less intense than C1q and C3 deposition, properdin deposition occurred in eight of the thirteen skin lesions tested but in only two of nine normal areas. C3PA deposition was of greater intensity than was properdin, but was seen in only five lesions and three clinically normal areas. Seven skin lesions and two normal areas also demonstrated fibrin deposition. Although alternate pathway activation is apparent, our findings suggest that the classical pathway (C1 to C9) is the primary complement pathway involved in SLE skin.

Since the initial reports by Burnham, Neblett & Fine (1963) and Cormane (1964) describing the deposition of immunoglobulins in cutaneous lesions and clinically normal skin of patients with systemic lupus erythematosus (SLE), numerous studies using direct immunofluorescent (IF) techniques have confirmed the earlier findings (Tan & Kunkel, 1966; Ten Have-Opbroek, 1966; Pohle & Tuffanelli, 1968). Later reports have stressed the importance of demonstrating the deposition of immunoglobulins by direct IF staining in uninvolved skin, as well as in skin lesions, as a useful diagnostic test for SLE (Pohle & Tuffanelli, 1968; Chorzelski, Jablonska & Blaszczyk, 1969; Burnham & Fine, 1971).

Other immunopathological investigations (Baart de la Faille-Kuyper & Cormane, 1968; Burnham &

Fine, 1969; Chorzelski *et al.*, 1969) have revealed dermal–epidermal deposition of complement (C3 and C4) in involved and uninvolved SLE skin, suggesting that the deposits of immunoglobulins represent antigen–antibody complexes. Since deposition of C4 occurs, in addition to C3 deposition, such complexes appear capable of activating the classical complement pathway (C1 to C9).

Recently, however, an alternate pathway of complement activation has been described (Götze & Müller-Eberhard, 1971; Ruddy, Gigli & Austen, 1972; Lachmann & Nicol, 1973) in which the early reacting complement components (C1, C4, and C2) are bypassed. In this pathway, now recognized to be the old properdin system (Pillemer *et al.*, 1954; Goodkofsky & Lepow, 1971; Götze & Müller-Eberhard, 1974) and referred to as the properdin pathway, the terminal complement components (C3 to C9) are activated by a separate system of serum factors that, in addition to properdin, include C3 proactivator (C3PA) and C3PA convertase (C3PAase). Properdin is now believed to initiate alternate pathway activation by interacting with C3PAase (Götze & Müller-Eberhard, 1974). This pathway may be activated by a factor found in cobra venom, inulin, zymosan, endotoxins, and aggregates of certain immunoglobulins (in particular IgA), which are not capable of classical pathway activation (Ruddy *et al.*, 1972; Lachmann & Nicol, 1973; Götze & Müller-Eberhard, 1974).

Recently, Rothfield *et al.* (1972) and Provost & Tomasi (1973) have demonstrated deposition of properdin in skin lesions of patients with SLE. The latter investigators further demonstrated the deposition of both C3PA and properdin in SLE skin lesions. Both studies suggest activation of the alternate or properdin pathway in SLE in addition to the classical complement pathway.

The present studies, therefore, were designed to evaluate these findings, to determine if deposition of properdin and C3PA occurs in skin lesions and in clinically normal skin of patients with SLE, and to test further the specificity of these two IF staining reactions.

METHODS

Thirteen patients with SLE were included in this study. All patients had active clinical disease when skin specimens were obtained, and with one exception (case 10, Table 1) all of the patients had other major clinical criteria for SLE as established recently (Cohen *et al.*, 1971). Clinical and pertinent laboratory data of our thirteen patients appear in Table 1. Patients with other dermatoses served as controls.

Diseased and clinically normal, sun-protected skin specimens were obtained by punch biopsy or surgical excision and were immediately frozen in liquid nitrogen. All specimens were stored at −70°C and were processed within 1 week. Specimens were sectioned in a cryostat at −30°C and were used unfixed in the various IF staining procedures.

Antisera monospecific for IgG were prepared, assayed, and conjugated with fluorescein isothiocyanate by established methodology (Beutner, Chorzelski & Jordon, 1970). Antisera monospecific for human IgA, IgM, β_1C/β_1A globulins (C3), and fibrin were purchased* and tested for specificity by both immunodiffusion (Ouchterlony) and immuno-electrophoresis. Fluorescein to protein ratios (F/P), units of antiserum, and use dilutions (U.D.) for these conjugated antisera are shown in Table 2.

Antiserum to human C1q, a gift of Dr Mark Ballow, was prepared in rabbits according to Morse & Christian (1964) and used at a 1:100 dilution. Rabbit antiserum to C3PA, a gift of Dr Noorbibi Day, was prepared and tested for specificity by the method of Götze & Müller-Eberhard (1971). Briefly, this antiserum was tested by immuno-electrophoresis against normal human serum as well as normal human serum with inulin added. As previously described (Götze & Müller-Eberhard, 1971), by adding inulin to normal human serum, a gamma-migrating precipitin arc was produced, in addition to the beta-migrating protein seen in normal human serum alone. This antiserum was used at a 1:20

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* Hyland Division, Travenol Laboratories, Costa Mesa, CA 92626.

dilution for IF testing. Antiserum to human properdin was made in a goat after isolation of properdin according to Pensky *et al.* (1968). Details for the preparation of this antiserum have previously been outlined (Jordon *et al.*, 1974). With immuno-electrophoresis, this antiserum demonstrated a cathodally migrating precipitin arc against purified properdin, similar to the pattern recently described by McLean & Michael (1972) for aged serum. To ensure its specificity, the anti-properdin antiserum was absorbed with 'RP' reagent also prepared according to Pensky *et al.* (1968). But to ensure that 'RP' reagent was completely free of properdin, it was absorbed twice more with zymosan.* This antiserum was used at a 1:10 dilution.

TABLE 1. Clinical and laboratory findings in thirteen cases of systemic lupus erythematosus

Case	Sex and age (years)	LE clot test	ANF	Sed. rate (mm/1 h)	CH_{50} units*	Arthritis	Nephritis	Other
1	F, 22	+	+	63	< 10	Present	Absent	Myasthenia gravis, leukopenia
2	F, 36	+	+	87	20	Present	Absent	Anaemia, pulmonary fibrosis
3	F, 40	+	+	20	32	Absent	Present	False-positive serology, leukopenia
4	F, 41	+	+	97	14	Absent	Present	CNS symptoms, leukopenia
5	F, 23	+	+	82	75	Absent	Absent	Fever, anaemia, pleuritis, Raynaud's disease
6	F, 60	+	+	54	34	Present	Absent	Hepatitis, pleuritis, CNS symptoms
7	M, 54	+	+	100	30	Present	Absent	(?) drug-induced, pleuritis
8	F, 35	Rosette formation	+	58	62	Present	Absent	False-positive serology, pericarditis, leukopenia, CNS symptoms
9	F, 54	+	+	110	44	Present	Absent	(?) drug-induced, leukopenia, Coombs' positive anaemia
10	M, 49	−	+	40	65	Absent	Absent	Disseminated discoid skin lesions only
11	F, 44	+	+	66	17	Present	Absent	Hashimoto's thyroiditis, leukopenia
12	F, 30	−	+	9	< 10	Present	Absent	Papilloedema
13	F, 40	+	+	97	< 10	Present	Present	Pleural effusions, leukopenia

* Total complement in CH_{50} haemolytic units.

Direct IF staining of diseased and clinically normal skin was performed by standard procedures (Beutner *et al.*, 1970) using labelled antisera to IgG, IgA, IgM, C3, and fibrin. The presence of C1q, C3PA and properdin was determined by a modified indirect IF technique recently outlined by Provost & Tomasi (1973). Briefly, sections treated initially with unlabelled rabbit anti-C1q or rabbit anti-C3PA were subsequently treated with labelled goat anti-rabbit IgG (F/P 4.4, use dilution 1:32).

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* Schwarz/Mann Division, Becton, Dickinson & Co., Orangeburg, NY 10962.

Sections treated with unlabelled goat antiproperdin were then treated with labelled rabbit anti-goat IgG (F/P 3.5, used dilution 1:32) as the second step of the procedure.

TABLE 2. Labelled anti-human antisera characteristics

Specificity of conjugates	F/P ratios*	Antibody assays		Dilutions for IF test
		AbP, mg/ml†	Units‡	
Anti-IgG	2·6	1·9	8	1:32
Anti-IgG	3·5	2·2	32	1:128
Anti-IgA	3·2	0·5	4	1:16
Anti-IgM	2·6	1·9	8	1:32
Anti-C3	3·5	1·6	8	1:40
Anti-C3	3·4	2·0	8	1:40
Anti-fibrin	2·2	Not available	Not available	1:16

* Ratio of molar fluorescein to protein.

† AbP (antibody protein).

‡ Units determined in gel diffusion (Ouchterlony) by standard methods (Beutner *et al.*, 1970).

TABLE 3. Summary of immunofluorescent studies of skin lesions and clinically normal skin in patients with SLE (total positives)

Antisera	Skin lesions	Normal skin
Anti-IgG	11/13	6/11
Anti-IgA	3/11	2/8
Anti-IgM	13/13	11/11
Anti-C1q*	13/13	7/9
Anti-C3	12/13	8/11
Anti-C3PA*	5/13	3/9
Anti-properdin*	8/13	2/9
Anti-fibrin	7/13	2/11

* Modified indirect immunofluorescent staining employed.

RESULTS

Results of direct IF (IgG, IgA, IgM, C3, and fibrin) and modified indirect IF staining (C1q, C3PA and properdin) of thirteen skin lesions and eleven clinically normal areas from patients with SLE are shown in Table 3. IgM was the predominant immunoglobulin at the dermal–epidermal junction, being present in all thirteen lesions (Fig. 1a) and all 11 normal areas. IgG, the next most common immunoglobulin, was detected in eleven lesions but in only 6 of the clinically normal areas. The intensity of IgM staining, however, was always greater than that of IgG. IgA was the least frequently encountered immunoglobulin, being present in small amounts in three of eleven lesions and two of eight clinically normal areas.

Factors associated with classical pathway activation of complement were present in all of the skin lesions and many of the uninvolved areas. Deposition of C1q occurred in all thirteen skin lesions (Fig. 1b) and in seven of nine clinically normal areas tested; two normal areas were not tested for C1q activity. Deposits of C3 were present in twelve of the thirteen lesions tested (Fig. 1c) and in eight of eleven normal-appearing areas.

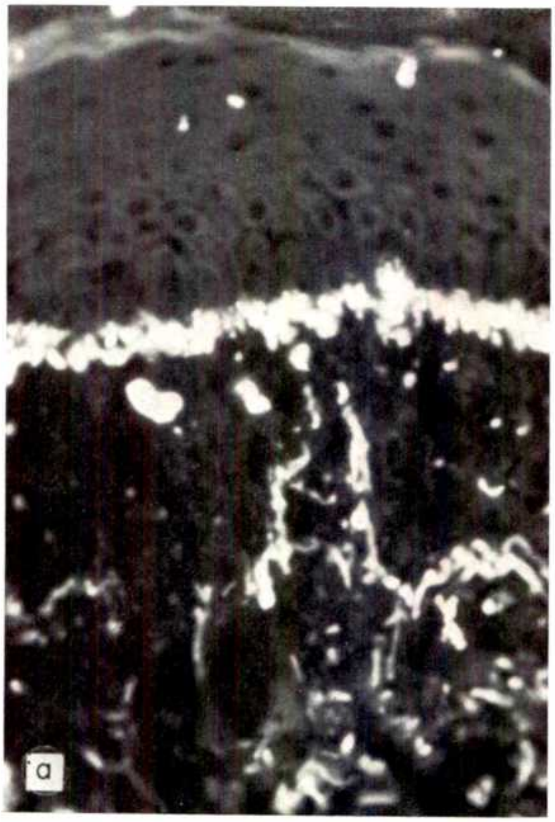

FIGURE 1a. Immunofluorescent staining of skin lesion of patient with systemic lupus erythematosus. Granular deposition of IgM at dermal–epidermal junction (× 250).

Although much less frequent than C1q and C3, factors associated with the alternate complement pathway were detectable in both diseased and clinically normal SLE skin. Deposition of properdin occurred in eight of the thirteen lesions tested (Fig. 2a) but in only two of the nine normal-appearing areas tested. The intensity of properdin staining, however, was considerably less than that of IgG, IgM, C1q, or C3 staining. Staining of C3PA, although considerably more intense than properdin staining, was seen in only five lesions (Fig. 2b) and in only three normal areas.

Deposition of fibrin was observed in seven of the skin lesions but in only two of the uninvolved areas.

Similar granular localization of complement components (C1q and C3), C3PA, and properdin at the dermal-epidermal junction was not seen in skin biopsy specimens of patients with other various dermatoses, including patients with vasculitis, pemphigus vulgaris, lichen planus, and datitiserm herpetiformis. Linear deposition of C1q, C3, C3PA, and properdin in bullous pemphigoid lesions previously noted by Provost & Tomasi (1973), has been described (Jordon *et al.*, 1974).

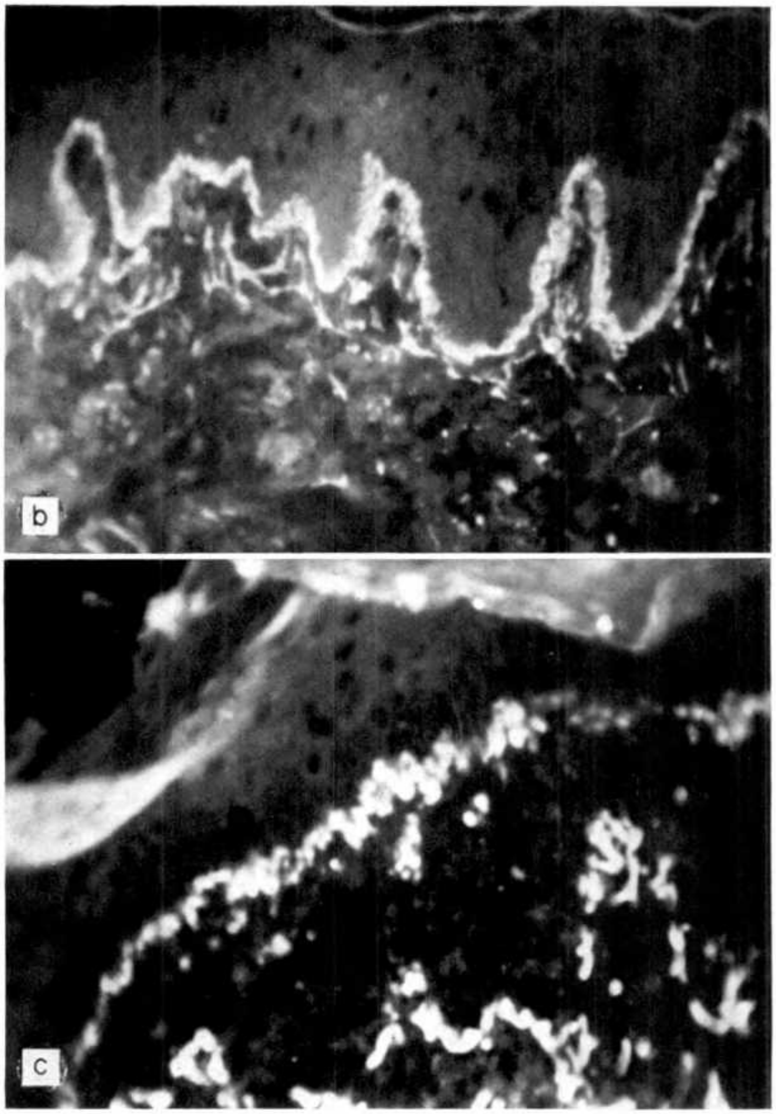

FIGURE 1b, c. Immunofluorescent staining of skin lesions of paitents with systemic lupus erythematosus. (b) C1q deposition (× 250). (c) C3 deposition (× 250).

Treatment of the SLE skin sections with either conjugated goat anti-rabbit or rabbit anti-goat IgG alone did not result in specific dermal–epidermal zone staining. Two earlier cases, however, where weak granular staining occurred using conjugated goat anti-rabbit serum, were eliminated from this study. Prior treatment of SLE skin sections with normal goat or rabbit serum did not result in staining. Finally, specific absorption of the anti-C3PA and antiproperdin with C3PA and properdin, respectively, resulted in blocking of the specific IF staining reactions. Similar blocking experiments demonstrating specificity have been carried out with bullous pemphigoid skin lesions in which heavy linear deposition of properdin occurred (Jordon *et al.*, 1974).

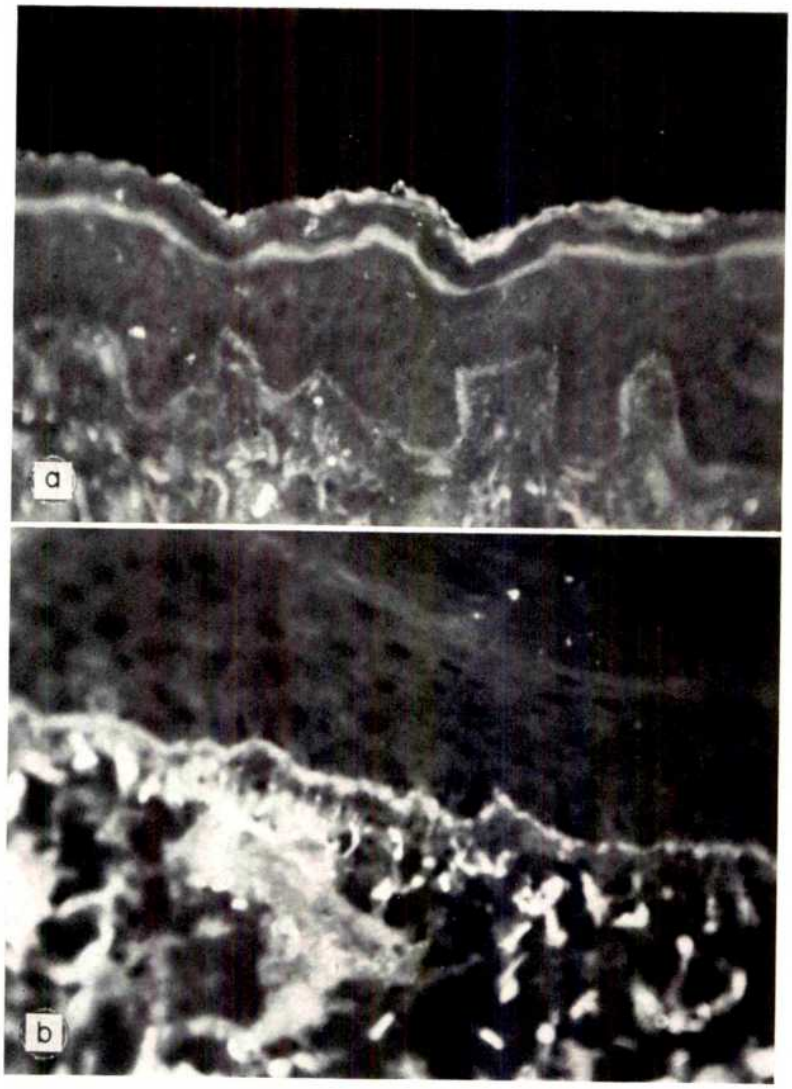

FIGURE 2. Immunofluorescent staining for alternate complement pathway factors in skin lesions of patients with systemic lupus erythematosus. (a) Properdin deposition (× 250). (b) C3PA deposition (× 250).

DISCUSSION

Although the small series of patients reported herein represents a selected study, all SLE patients with deposition of immunoglobulins in skin lesions also had similar deposition in clinically normal skin, a finding consistent with many previous observations (Cormane *et al.*, 1966; Pohle & Tuffanelli, 1968; Burnham & Fine, 1971). Unexpectedly, IgM, rather than IgG, was the predominant immunoglobulin encountered, and in almost all instances IgM staining was considerably more intense than IgG staining. Ten Have-Opbroek (1966) and Pohle & Tuffanelli (1968) have made similar observations. Other investigators, however, believe that IgG represents the predominant immunoglobulin (Cormane *et al.*, 1966; Tan & Kunkel, 1966; Burnham & Fine, 1969). Further studies of this discrepancy seem warranted.

The nature of the IgM deposits also needs clarification. Recently, Landry & Sams (1973) eluted anti-nuclear factor (ANF) from two of three SLE skin specimens by acid-elution techniques. The eluates contained weak ANF of the IgM type. Since this eluted IgM ANF was weak when compared to eluted IgG ANF, one wonders if other factors, such as rheumatoid factors or cryoglobulins, might not also deposit at the dermal-epidermal junction. These possibilities are being investigated further.

Deposition of complement was noted in all of the tissue studies. C1q was the predominant component found and was present in all thirteen lesions studied, although deposition of C3 was present in twelve. Deposition of C1q and C3 also occurred in a high proportion of the clinically normal areas, seven of nine and eight of eleven, respectively. These findings suggest involvement of the classical complement pathway in both diseased and clinically normal SLE skin. At the same time, the findings add further credibility to the hypothesis that these deposits of immunoglobulins represent immune complexes. Further support for this concept has been documented recently by Landry & Sams (1973), who successfully eluted complement-fixing ANF from SLE skin. They further observed a complement-fixing basement-membrane antibody, similar to that found in bullous pemphigoid (Jordon, Sams & Beutner, 1969), in skin eluates. This basement-membrane antibody was IgG, however, rather than IgM.

Deposition of fibrin occurred in a few lesions and clinically normal areas, particularly if clinical activity had been present for some time. Whether such deposition is the result of complement activation (Ruddy *et al.*, 1972) or of some other unrelated mechanism activating the clotting sequence must be investigated further.

Alternate pathway factors, that is, C3PA and properdin, were found less frequently than were C1q and C3. Although present in eight of the thirteen lesions tested, properdin was seen in only two of nine clinically normal areas. Properdin staining was always less intense than C1q or C3 staining. These findings contrast somewhat with our recent observations in bullous pemphigoid (Jordon *et al.*, 1974) in which heavy staining of properdin occurs in addition to C1q and C3 staining.

Deposition of C3PA occurred less frequently than did properdin deposition, and often was independent of properdin deposition. Whether this reflects 'C3b' feedback activation of the alternate pathway, as outlined recently by Lachmann & Nicol (1973) and Götze & Müller-Eberhard (1974), must be investigated further. Provost & Tomasi (1973) also found deposition of C3PA and properdin in only a few of their patients (five of twenty-five) and then only in skin lesions. C1q was present in these same patients, but whether C1q was present in the remaining patients was not reported. We can only surmise from our data, therefore, that alternate pathway activation of complement appears to be secondary to classical pathway activation. But whether classical or alternate pathway activation of complement, or both, contributes to the production of SLE skin lesions must still be resolved.

ACKNOWLEDGMENTS

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Tumour angiogenic factor associated with subcutaneous lymphoma

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SUMMARY

Growth and survival of cutaneous neoplasms are dependent on the development of a nutrient-supplying vascular lifeline. A subcutaneous lymphoma is described which mimicked a vascular neoplasm because of the extensive telangiectasia overlying the tumour. Fragments of the lymphoma inserted in direct apposition to the hamster cheek pouch membrane or separated from the membrane by a microporous filter both induced dramatic neovascularization. Control materials failed to induce significant vascular changes. A diffusable Tumour Angiogenic Factor may play a vital role in tumour survival and portends a therapeutic potential.

As neoplastic cells proliferate and expand into a distinct tumour mass, their nutritional needs soon exceed the limits of interstitial fluids. It is then essential for the growing tumour that an adequate blood supply should be established. Ide, Baker & Warren (1939) and Algire & Chalkley (1945) have suggested that tumours are capable of meeting this Darwinian challenge by indirect stimulation of neovascularization in the host's vascular plexus. Goodall, Sanders & Shubik (1965) and Greenblatt & Shubik (1968) have subsequently demonstrated neovascularization with tumour implants in the hamster cheek pouch; Folkman *et al.* (1970) have isolated a diffusable Tumour Angiogenic Factor (TAF).

In this report we document, clinically and experimentally, the elaboration of a TAF by a malignant subcutaneous lymphoma.

CASE REPORT

A 4-year-old Oriental–American girl presented with a 2·0 × 3·0 cm subcutaneous mass in the centra forehead. The lesion had been noted 4 months earlier after a traumatic injury and had slowly increased in size; it was firm, moveable and non-tender. The most striking clinical feature was dramatic erythema and telangiectasia overlying the lesion. The appearance was that of a vascular tumour and the initial clinical differential diagnosis included haematoma, haemangioma and haemangio-endothelioma.

An excisional biopsy was performed because of the increasing size of the lesion and a lobulated, non-encapsulated tumour was removed and submitted for routine histopathology. Sections of tumour stained with haematoxylin and eosin revealed densely-packed dermal masses of small deeply basophilic cells extending into the subcutis (Figs. 1 and 2). The histopathological diagnosis was 'malignant lymphoma, undifferentiated, non-Burkitt type.'

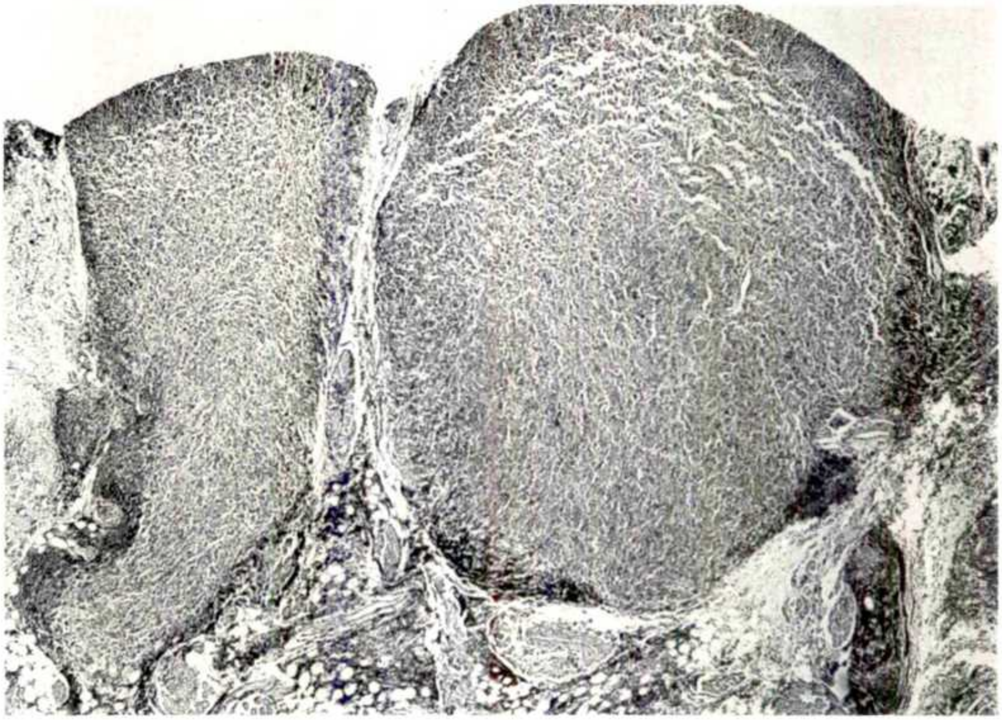

FIGURE 1. Dermal nodules of basophilic cells extending into the subcutis diagnosed as subcutaneous lymphoma (H & E, × 44).

Investigations

Physical examination was completely normal with the exception of the above-mentioned fronto-glabellar mass. The white blood cell count was 18,900/mm^3, with 40% lymphocytes and 5% monocytes. Other laboratory findings were entirely within normal limits including the following: chest X-ray, skull X-ray, complete radiological bone survey, intravenous pyelogram, bone marrow aspiration, haemoglobin, haematocrit, platelet count, blood urea nitrogen, fasting blood glucose, and liver function tests.

Since the white cell count was elevated and this type of lymphoma is often considered pre-leukaemic, the patient received a course of radiation therapy to the tumour site combined with systemic chemotherapy (vincristine, methotrexate and prednisone). Six months after surgery the patient is in good health with no signs of local tumour recurrence or systemic illness.

Demonstration of TAF

Fragments of the surgically excised lymphoma were transported to the laboratory in refrigerated normal saline and immediately implanted into hamster cheek pouch chambers by the classical tech-

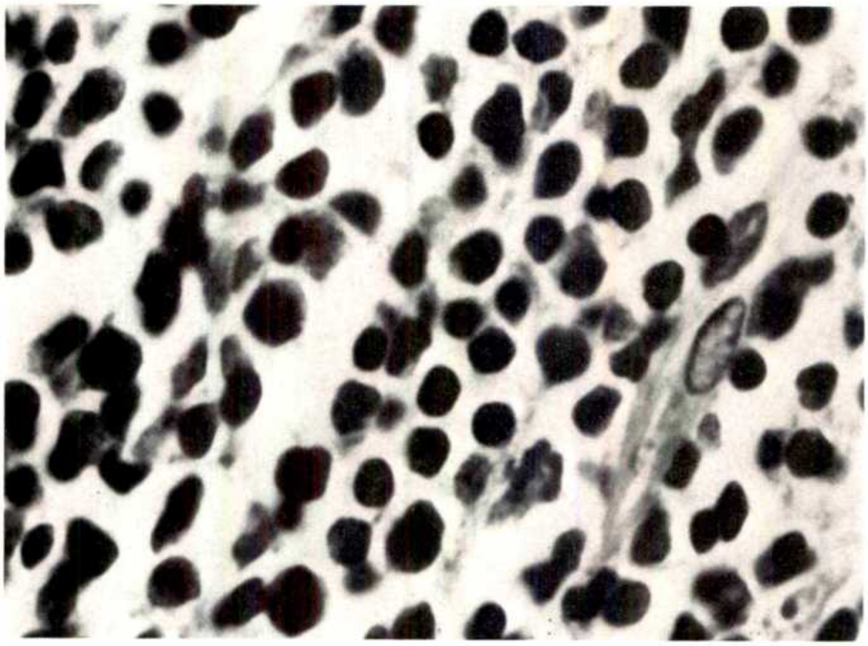

FIGURE 2. The deep dermal nodules of the excised tumour were composed of deeply basophilic lymphocytes.

nique of Sanders & Shubik (1964), Greenblatt and Shubik (1968) and Warren and Shubik (1966). The immunologically 'privileged' domain of the hamster cheek pouch allowed us to observe the effect of tumour implants on small blood vessels in an anatomically and physiologically normal setting.

In six hamsters, 2·0 mm tumour fragments were implanted in direct apposition to the vascular membrane of the cheek pouch. In an additional six hamsters, similar implants were separated from the cheek pouch vascular bed by 6·0 mm discs of millipore filter (Type TA, 0·45 μm pore size, 25 μm thick) in order to test for the presence of a diffusable Tumour Angiogenic Factor. Implanted materials were observed for 7–14 days with a stereoscopic binocular microscope and with an American Optical microscope with long distance objectives. The vascular response of the cheek pouch membrane to tumour grafts was contrasted with that to normal human dermis, keloid, naevocellular nevi and inert control materials (Millipore filter, dialysis membrane).

Lymphoma grafts stimulated neovascularization in the hamster cheek pouch directly and across a microporous membrane (Millipore) barrier. The usual sequence of vascular events consisted of arterial tortuosity and vasodilation, followed by early sprouting of tiny capillary buds, overgrowth of implants and establishment of complex functioning vascular arcades (Fig. 3). Controls showed occasional tortuosity and dilatation without angiogenesis.

DISCUSSION

Greenblatt & Shubik (1968), Goodall *et al.* (1965) and Warren & Shubik (1966) demonstrated a diffusable, angiogenically active substance in the hamster cheek pouch with direct tumour implants and transfilter diffusion studies. Folkman *et al.* (1970) have subsequently isolated a Tumour Angiogenic Factor and characterized it as a heat-labile, cryostable lipoprotein containing 25% RNA and 50%

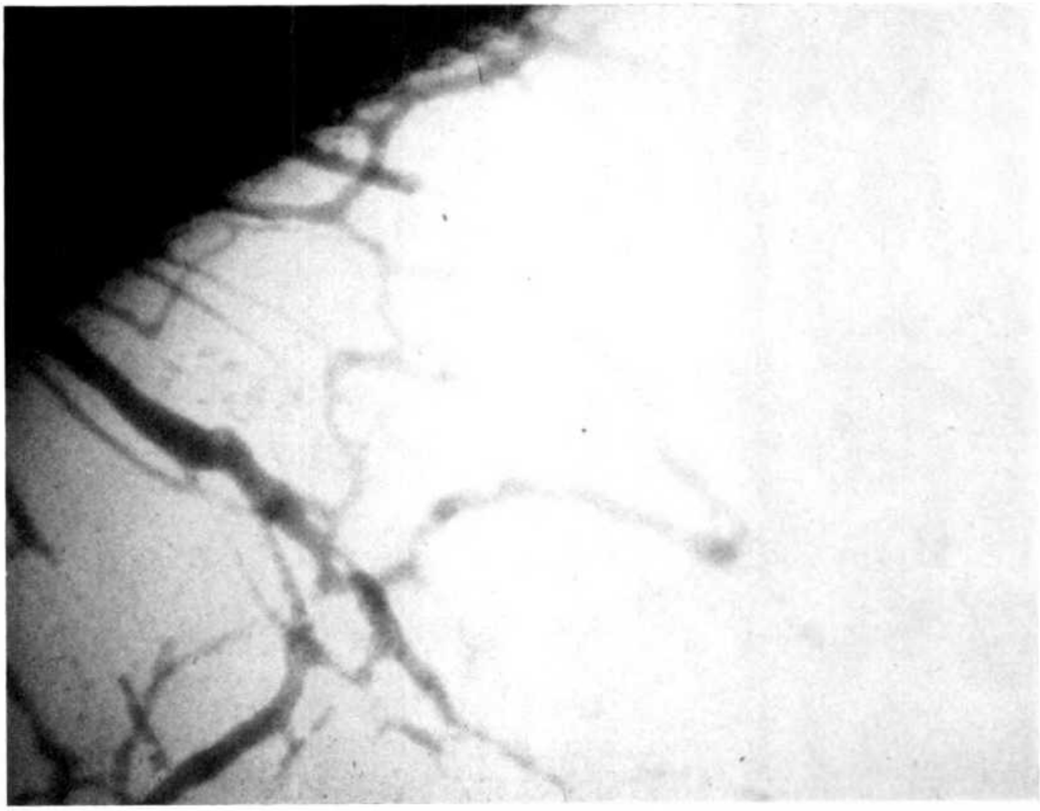

FIGURE 3. Fragments of the subcutaneous lymphoma elicited the formation of reactive neovascular arcades in the hamster cheek pouch membrane across a Millipore filter (× 100).

carbohydrate. The exact mechanism of action of TAF is not entirely clear, but Warren, Greenblatt & Kommineni (1972), Folkman *et al.* (1970) and Cavallo *et al.* (1972) offered evidence that the factor is directly mitogenic for capillary endothelial cells.

In this report we have demonstrated the production of TAF by a malignant subcutaneous lymphoma in order to italicize the immediate and potential dermatological implications of this angiogenic factor. The tumour reported was initially thought to be a blood vessel neoplasm when presented at a university teaching conference, thus emphasizing the clinically vascular appearance of the lesion. Human skin tumours are often associated with alteration of cutaneous vessels—for example the telangiectasia seen with basal cell epithelioma, subcutaneous medulloblastoma and metastatic carcinoma (Primack, 1973). These diagnostically significant vascular changes may be due to capillary proliferation induced by a Tumour Angiogenic Factor.

There is also a chance that TAF may one day assume a therapeutic potential. Tannock (1968) has demonstrated that the expansion of a tumour mass is directly limited by its blood supply and Folkman *et al.* (1970) have suggested that tumour growth might be arrested by interfering with the elaboration and/or function of TAF. If so, cutaneous neoplasms, exquisitely visible and eminently accessible, might provide an excellent proving ground for the theory.

In this report, we have utilized laboratory techniques to confirm a clinically relevant concept with widespread implications for dermatology.

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Psoriasiform napkin dermatitis—a follow-up study

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SUMMARY

One hundred and twenty-three children who had napkin dermatitis, with or without a secondary sensitization eruption, in infancy were reviewed 5–13 years later. Of the seventy-one who had a predominantly psoriasiform secondary eruption, twelve (17%) had psoriasis at review—three (4%) had atopic eczema. None of the forty treated for a predominantly seborrhoeic secondary eruption had psoriasis at review—15 (37%) had atopic eczema. The psoriasiform group had the highest incidence of psoriasis and the lowest incidence of atopy among first degree relatives. The converse incidence was found in the seborrhoeic group.

It is suggested that infants who develop psoriasiform napkin dermatitis have a psoriatic diathesis.

Most dermatologists accept a distinction between napkin dermatitis (erythematous, erosive, bullous or scaling) localized to areas of maximum contact with the napkin and those confluent dermatoses involving the whole napkin area including the body folds within the napkin area. Both types of napkin dermatitis can be associated with a generalized eruption although this occurs more frequently with the intertriginous type. The onset of the secondary eruption is often 'explosive' and the morphology varies between a psoriasiform eruption and seborrhoeic dermatitis of infancy. Both types of secondary eruption may become almost generalized and are often associated with marked crusting of the eyebrows, forehead and scalp.

The term 'napkin psoriasis' was used to describe the psoriasiform type of eruption by Warin & Faulkner (1961). Warin (1967) later reported that he had observed seven children who developed typical psoriasis a few years after presenting with napkin psoriasis. Fergusson, Fraser & Grant (1966), Jefferson (1967) and Meara (1968), although recognizing the psoriasiform picture, favoured the retention of this eruption within the seborrhoeic grouping.

Jefferson (1966) observed one child who developed atopic eczema after the initial napkin dermatitis with sensitization eruption. Andersen & Thomsen (1971) showed that psoriasis and a positive family history of psoriasis were more common in children who had suffered from psoriasiform napkin dermatitis. However, they also found that 6·6% of their patients had seborrhoeic eczema at review.

In an attempt to clarify the position, this report presents the results of review examination of 123 children treated in infancy for severe napkin eruptions. At the time of review the children's ages ranged from 5–13 years.

METHOD

The case records of all children under the age of 2 years treated for severe napkin eruptions from 1960–67 by the staff of this department were reviewed. This review included the fifty-two children reported by Fergusson *et al.* (1966). Where the secondary eruption was predominantly psoriasiform, the condition was labelled as 'psoriasiform napkin dermatitis' (PND) and where the secondary eruption affected mainly the flexures, the condition was labelled 'seborrhoeic napkin dermatitis' (SND). Some secondary eruptions showed mixed and varying features and were impossible to ascribe to a particular group and were classified as 'generalized napkin dermatitis' (GND). If the eruption was localized to areas of maximum contact with the napkin, the condition was diagnosed as 'napkin dermatitis' (ND). Children diagnosed as suffering from infantile eczema or atopic eczema during the initial period of attendance with napkin dermatitis were excluded from the review. In addition, one child originally diagnosed as psoriasiform napkin dermatitis was also excluded because the diagnosis was altered to psoriasis during her attendance in infancy. It is of interest to note that she had been clear of skin disease for 7 years when she was seen for review at the age of 11 years.

Employing these rather simple clinical criteria, the patients were allocated to the following groups:

PND 118 patients (51 males: 67 females)
SND 96 patients (56 males: 40 females)
GND 25 patients (8 males: 17 females)
ND 32 patients (13 males: 19 females)

A letter was sent to the mother of each of these 271 children, giving the child an appointment at the outpatient clinic. The mother was asked to determine if there was a family history of skin disease.

Only 123 children attended for review. The case records of those who failed to attend were scrutinized and a note was made of any referral to this department after the initial episode of napkin dermatitis. Five children, classified originally as SND, were referred later to this department with skin disease. Four (aged 18, 20, 27 and 33 months) were suffering from atopic eczema and another aged 5 years was found to have seborrhoeic eczema. These children have been included as reviewed.

At follow-up interview the children were examined for skin disease and a parent questioned for a family history of asthma, eczema or hay-fever (atopy) or of psoriasis. Affected relatives were recorded as first degree (parents and siblings), second degree (aunts, uncles and grand-parents) and third degree (cousins).

A control group of sixty-five children attending for treatment of warts was studied in the same manner.

RESULTS

Age-of-onset

In the majority of cases the secondary eruption was alarming to the parents, and the infants were seen at hospital within a few days of its onset. Thus this age was regarded as the 'age-of-onset'. From Fig. 1 it can be seen that 65·6% of the 118 children suffering from PND attended hospital before they were 100 days old. Only 30% of the 96 infants with SND (Fig. 2) were seen by that age. The infants with GND and ND (Figs. 3 and 4) were more evenly spread over the first year of life.

Results of examination

Of the 113 children examined, forty-three had skin lesions. The type of eruption and the average age at the time of review are shown in Table 1.

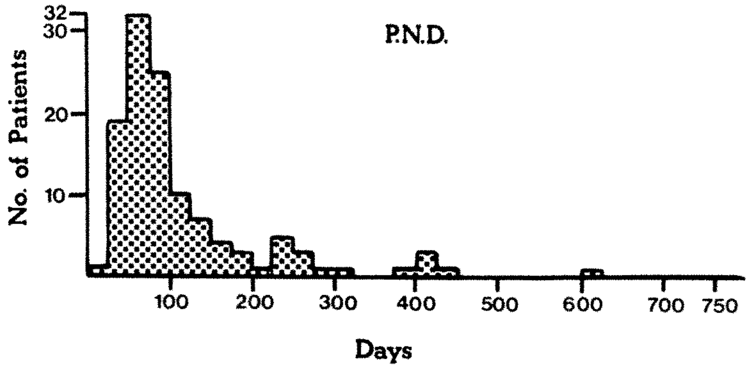

FIGURE 1.

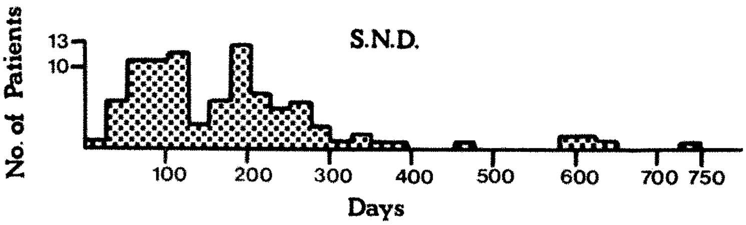

FIGURE 2.

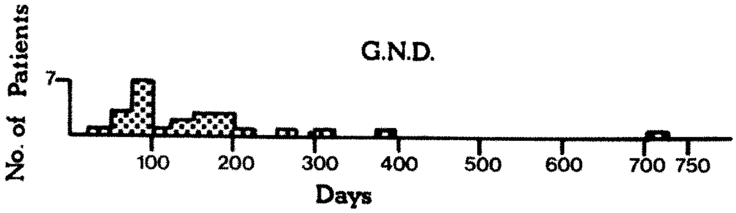

FIGURE 3.

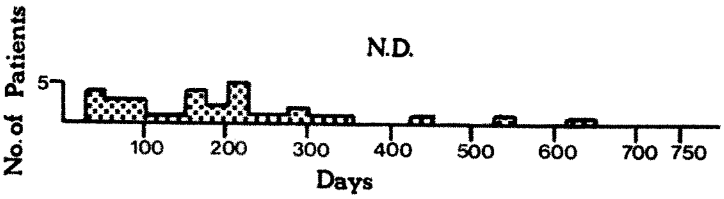

FIGURE 4.

FIGURES 1.–4. Age of onset of eruption in different groups of children with napkin dermatitis.

Psoriasiform napkin dermatitis group

Of the seventy-one children in this group there were—

(a) Seven patients with psoriasis of limbs and trunk
(b) Five girls with psoriasis of scalp only
(c) Four children with 'scaling lesions'

(a) *Psoriasis.* Seven children (three boys and four girls) had typical lesions of psoriasis on their limbs and trunk without scalp involvement. Four of them (aged 7, 7, 8 and 11 years) had typical plaques of psoriasis. Histology from one of these patients showed the characteristic features of psoriasis. Two girls (aged eight and nine years) each had a solitary, typical, guttate lesion of psoriasis on the anterior trunk. When the younger girl was seen again 1 year later, the original lesion had disappeared spontaneously and a new, similar lesion had developed. At the second visit she was found also to have three 1·0 cm diameter 'scaling lesions' over the sacrum. These 'scaling lesions' had been observed also in one of the children who had plaques of psoriasis. The seventh child in this group, a boy aged 8 years, was regarded by us as having psoriasis. His lesions were superficial rather than heaped, they persisted for 16 months, he had pitting of the finger-nails, he developed a Köbner reaction at the site of a biopsy, and the lesions cleared after the application of 2% tar ointment for 3 weeks. The histology of the small lesion removed showed only perivascular infiltration.

TABLE 1. Findings at review examination

Type of eruption	Total reviewed	Average age (years)	Psoriasis	Scalp psoriasis	Scaling lesions	Eczema	Other skin disease
PND	71	7·8	7	5	4	5	Finger-nail pitting (2) Patch of hyperkeratosis (1) Seborrhoea capitis (1) Angular perlèche (1)
SND	40	8·3	0	0	0	16	Xeroderma (1)
GND	2	12·0	No skin disease at review examination (apart from warts in control group)				
ND	10	7·4					
Controls	65	8·5					

(b) *Psoriasis of Scalp.* Five girls (aged 7, 7, 7, 9 and 11 years) had psoriasis of the scalp without involvement of the trunk or limbs. One girl had a single, heaped, scaling lesion 2·0 × 3·0 cm and another had two similar lesions. A third girl was seen in another area at our request by a dermatologist who reported 'one patch of psoriasis on her scalp'. These three girls were seen on only one occasion. Two girls had recurrent, heaped, scaling lesions at review over 1 year. As one lesion healed, another appeared. At her third visit, one of these girls also had seven 'scaling lesions' on her lower abdomen and thighs. Histology from one of her scalp lesions suggested seborrhoeic dermatitis rather than psoriasis. However, despite the histology report, from the clinical appearances it was felt that these were all examples of mild scalp psoriasis in childhood.

(c) *Scaling Lesions.* Three boys (aged 7, 8 and 8 years) and one girl (aged 8 years) had this type of lesion without any other manifestation of psoriasis. It was noted above that two patients with psoriasis on the body and limbs and one with scalp psoriasis had this type of lesion in addition to their more obviously psoriatic eruption. The lesions were scaling, flat and yellowish-brown on a pink base. The scale was difficult to remove by scraping and lacked the usual silvery appearance found in psoriasis. Punctate haemorrhage did not result from scraping off the scale. These lesions varied from small, solitary or grouped lesions 0·2–0·3 cm diameter to plaques up to 2·0 × 3·0 cm. They were found on the trunk, proximal parts of the limbs and in one child on the shaft of the penis.

A variety of skin conditions was found in another 10 children. Two had minimal transient pitting of the finger nails. Three had typical atopic eczema and one had mild seborrhoeic eczema. A transient eczematous patch, angular perlèche, seborrhoea capitis and a transient hyperkeratotic (non-scaling) lesion were each found in one child.

Seborrhoeic napkin dermatitis group

None of the forty children in this group was found to have psoriasis or psoriasiform lesions. However, fifteen had atopic eczema, one had xeroderma and one had seborrhoeic eczema at review. Of the fifteen children with atopic eczema, four had chronic flexural eczema, nine had continuing, active, extensive eczema and two had mild eczema. Three of these children (one from each sub-group) had asthma.

Generalized napkin dermatitis, napkin dermatitis and control groups

None of these children was found at review to have skin disease (other than warts in the control group).

TABLE 2. Incidence of psoriasis and atopy in relatives

		First degree Relatives				First and second degree Relatives				First, second and third degree Relatives			
		Psoriasis		Atopy		Psoriasis		Atopy		Psoriasis		Atopy	
Type of eruption	Total	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
PND	70	5	7·15	13	18·6	12	17·2	22	31·5	13	18·6	24	34·3
SND	33	0	0	11	33·3	3	9·1	13	39·4	4	12·1	14	42·4
Controls	65	2	3·1	18	27·7	10	15·4	27	41·6	10	15·4	34	52·4

Family History

It can be seen from Table 2 that 7·15% of the PND children had a parent or sibling affected by psoriasis, whereas none of the SND group and only 3·1% of the control group had a first degree relative affective by psoriasis. The highest incidence of atopy in first degree relatives was found in the SND group. The GND and ND groups have not been recorded in Table 2 because of the small number in each group.

DISCUSSION

In this follow-up study, 113 children with widespread secondary eruptions associated with napkin dermatitis were reviewed 5–13 years later and forty-three (38%) were found to have skin lesions. When the secondary eruptions are classified according to the predominant clinical features into psoriasiform and seborrhoeic napkin dermatitis, the incidence of psoriasisis and atopic eczema at review is striking.

In the psoriasiform napkin dermatitis group of seventy-one children, twelve (16·9%) had psoriasis and, if the four children with 'scaling lesions' are included, 22·6% had psoriatic lesions at review. The average age of this group was only 7·8 years and further review at a later age is likely to produce a higher incidence of psoriasis.

It is of interest that none of the parents of the children found to have psoriasis at review knew that their child had psoriasis. In fact, the parents of one of the children with psoriasis and three of the children with scaling lesions were not aware of the lesions found at review. The failure of parents to observe these lesions limits the value of a postal questionnaire in this type of survey.

Most authorities quote an incidence of 1–2% for psoriasis in the Northern Hemisphere. Hellgren (1967) found an incidence of 1·86% in the general population of areas of Sweden and observed that 18% of psoriatics were aware of the disease starting in the first decade. Watson & Farber (1971) showed that 12% of psoriatics developed their disease by this age. Using these figures for a group of children under 13 years, an incidence of 0·4% for psoriasis could be expected, whereas the incidence of psoriasis in our PND group was at least 16·9%. If the 12 patients with psoriasis in the PND group are compared with the absence of psoriasis in the SND, GND, and ND groups taken together, our results are highly significant ($P<0{\cdot}005$).

The patients with scaling lesions present a diagnostic problem. They do not have the typical, heaped scaling lesions found in psoriasis. Watson & Farber (1971) stated that in children the lesions of psoriasis are thinner, softer and less scaly than those in adults. We suspect these minimal scaling lesions are an early manifestation of psoriasis in this group.

In sharp contrast to the incidence of psoriasis in the PND group, 27·5% of the SND group was found to have atopic eczema at review. Brereton *et al.* (1959) showed an incidence in school children of 0·98% for atopic eczema at 5 years, decreasing to 0·44% at 11 years. A higher incidence of 3·1% was observed by Walker & Warin (1956) in younger children. They considered that the true incidence of eczema might well be greater. Our results suggest a strong atopic tendency in those who develop SND in infancy.

Our analysis of the age of onset of the different types of napkin eruptions confirmed the findings of Warin & Faulkner (1961) and Fergusson *et al.* (1966). Most (65·6%) of the PND infants received hospital attention before they were 100 days old. The SND infants presented more evenly from the 25th to the 300th day of life. This suggests two distinct groups of infants developing their eruptions at different ages.

It is generally accepted that psoriasis has a genetic basis. Hellgren (1967) and Watson, Cann & Farber (1971) published results noting the exact relationship of the affected relative to the patient with psoriasis. Hellgren quoted a figure of 9·99% and Watson *et al.* 10·6% for first degree relatives affected by psoriasis. Their figures for control probands were 2·1% and 4·4% respectively (c.f. our control figure of 3·1%). Our results show that 7·15% of the PND children had a parent or sibling affected by psoriasis whereas none of the SND children had a psoriatic first degree relative. On the other hand the PND children had the lowest, and the SND children the highest incidence of atopy among first degree relatives.

These results show that the majority of infants with PND develop their eruption in the first 100 days of life, they have a high incidence of psoriasis and psoriasiform lesions 5–13 years later and, in addition, they have a strong family history of psoriasis. These findings suggest that psoriasiform napkin dermatitis is a definite entity, related in some way to psoriasis.

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We wish to express our thanks to the Consultant Dermatologists of this Department for permission to study children under their care. We are grateful to Dr N.G.Fraser for seeing one of the children in Aberdeen and to Dr R.S.Chapman for seeing another child for us and for helpful advice. We are indebted to Professor J.A.Milne for reviewing the histology. We wish also to thank Miss A.Dow for secretarial assistance.

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Nickel dermatitis hazards from prostheses

IN VIVO AND *IN VITRO* SOLUBILIZATION STUDIES

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SUMMARY

This report reviews data relating to the nickel hazard from implanted prostheses. It is shown that nickel is released from stainless steel prostheses by the action of sweat, blood and physiological saline solution. In laboratory animals, solubilized nickel is localized in the tissue near the implant.

Nickel-containing alloys have been implanted in man and in animals in a variety of therapeutic prostheses and devices. The most common alloys are those of stainless steel and cobalt–chromium (such as Vitallium). The stainless steel prostheses usually contain nickel in concentrations of 10–14%, but can contain concentrations as high as 35% (in the wire and probe leading to the heart muscle from a nickel–cadmium battery pacemaker). Common cobalt–chromium alloys for casting contain up to 2·5% nickel, while wrought alloys contain 9–10%.

In recent reports in the literature, instances of allergic reactions, urticarial (McKenzie, Aitken & Ridsdill-Smith, 1967; Symeonides, Paschaloglou & Papageorgiou, 1973) and eczematous dermatitis (Foussereau & Laugier, 1966; Tinckler, 1972; Brendlinger & Tarsitano, 1970; Barranco & Soloman, 1972; Pegum, 1974) to nickel have been attributed to implanted prostheses. Patch tests with nickel were positive in the patients with eczematous dermatitis. Removal of the metallic devices resulted in complete resolution of urticaria and involution of the eczemas. This would imply that nickel released from the metallic devices produced the allergic reaction in nickel-sensitive patients; a tenable thesis inasmuch as Ferguson *et al.* (1962) and Mears (1966) have reported increased nickel concentrations in parenchymal tissues from implantation of stainless steel rods containing nickel. Furthermore, Cohen (1962) has shown that corrosion of both stainless steel and cobalt–chromium alloys in simulated *in vivo* conditions is enhanced by cyclic stresses, such as are encountered in the use of weight-bearing orthopaedic implants. That this phenomenon does not occur in all such patients is noted in the case report by Lyell & Bain (1974). In their patient with proved nickel sensitivity, a Bjork–Shiley prosthetic

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valve (containing 300 mg of nickel) was used to replace the patient's diseased mitral valve. No adverse reactions were observed after a 22-month follow-up. However they do not dismiss the possibility that a reaction may arise especially as the valve is subject to constant trauma, and this may over a long period release significant quantities of metal. Pegum (1974) pointed out the importance of time; in his patient, who had a stainless steel plate inserted in the hip, all went well for 3 years, and then dermatitis appeared on various parts of the skin, and sterile suppuration developed around the plate.

Fisher (1972), commenting on the report by Barranco & Soloman (1972), rejected the possibility of such reactions on the grounds that nickel is so firmly bound physically in the alloy that body fluids and perspiration cannot 'leach' out the nickel to make it available to produce an allergic reaction. Fisher, however, has not considered the possibility of corrosion from implants. Samitz & Klein (1973) pointed out that corrosion of stainless steel orthopaedic prostheses has been well established (Cohen & Hammond, 1959; Scales, Winter & Shirley, 1961), and they suggested further investigation to determine if nickel is released from these materials. The variability of patient response and the variation in the detection of nickel reported here reflect the number of factors that affect corrosion *in vivo* including pH, PO_2, local stresses, and the presence of organic accelerating or passivating agents.

We have recently completed a laboratory investigation of the solubilization of nickel from stainless steel by sweat, whole blood, blood plasma, and physiological saline solution (PSS), and we have completed a small scale study of the translocation of nickel from implanted stainless steel to the surrounding tissue in laboratory animals. Several stainless steels, prostheses and surgical accessories were evaluated.

MATERIALS AND METHODS

Prostheses, suture wires, and screws were obtained from several manufacturers, and stainless steel samples were obtained through commercial sources. Large items were cut to a size that could be immersed in no more than 10 ml of 'leaching fluid' (sweat, blood, blood plasma or PSS). The test items, having surface areas of from 2 cm^2 to 5 cm^2, were washed in acetone and rinsed in distilled water. These items were then immersed in measured volumes of either sweat, blood, blood plasma, or PSS (contained in covered 50 ml polycarbonate beakers) for 1 week at room temperature (20–22°C). After this time, the liquid phases were analysed for nickel spectrophotometrically (Milner, 1963). The results of these measurements are summarized in Table 1.

Animal experiments

Incisions were made in both hind legs of each of five rabbits with a quartz scalpel. Stainless steel spheres were placed in the incisions in three animals, and polyethylene spheres were implanted as controls in the other two animals. The incisions were closed with nylon sutures. Three weeks later, biopsies were taken from the right leg of each animal with a quartz tube at the site of implant and 1, 2, 3, 4 and 5 cm from it. Biopsies were taken from the left side in the same manner 6 weeks after the implant. The biopsies were sealed in polyethylene tubing and analysed for nickel by neutron activation. These analyses were carried out at the University of Reading utilizing the facilities of the University of London Reactor Centre (ULRC). By careful control of the activation and cooling times, and by carrying out the activations with cadmium shielding, selective activation of nickel involving the ^{58}Ni (n, p)^{58}Co reaction was possible. Specific measurement of the ^{58}Co 810 KeV photopeak was made using the high-resolution Ge (Li) detector and 8196 channel analyser system at ULRC. Analysis of the data was by computer programme. The results of these measurements are listed in Table 2.

RESULTS

TABLE 1. Solubilization of nickel from stainless steel

Material tested	Nickel found in PSS, p.p.m.	Nickel found in sweat, p.p.m.	Nickel found in whole blood, p.p.m.	Nickel found in blood plasma, p.p.m.
302 s/s*	N.D.†	0·3		
303 s/s*	1·1	10·2		
316 L s/s	N.D.	N.D.	N.D.	N.D.
'Zimaloy' (Co–Cr type alloy)	0·1			
'Zimmer' 316 LVM	0·1			
Zimmer type 472 Jewett nail and plate	6·0	99·0	6·6	N.D.
	9·8	9·0	2·6	
Zimmer type 962 Jewett osteotomy plate	5·5	24·8	17·4	N.D.
'T & C' bone plate	1·8	12·1	15·3	1·0
Vitallium plate	0·6	7·2		
bone screw	3·0		N.D.	
suture wires (probably 316 L s/s)	0·5	0·6		
	0·7	N.D.		
Knowles pin	N.D.		N.D.	N.D.
Steinman pin	N.D.		N.D.	N.D.
Rush pin	N.D.		N.D.	N.D.

* Stainless steel from commercial channels (currently not used in prostheses)

† N.D., none detected, limit of detection ca. 1·0 μg

TABLE 2. Distribution of nickel in tissue near implanted stainless steel

Material implanted	Ni p.p.m. at distances indicated (cm) 0	1	2	3	4	5
after 3 weeks						
1 V4A s/s	46·2	N.D.†	N.D.	N.D.	N.D.	N.D.
2 V4A s/s	34·3	N.D.	N.D.	N.D.	N.D.	N.D.
3 V4A s/s	39·8	N.D.	N.D.	N.D.	N.D.	N.D.
4 polyethylene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5 polyethylene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
after 6 weeks						
1 V4A s/s	53·3	N.D.	N.D.	N.D.	N.D.	N.D.
2 V4A s/s	46·2	N.D.	N.D.	N.D.	N.D.	N.D.
3 V4A s/s	49·7	N.D.	N.D.	N.D.	N.D.	N.D.
4 polyethylene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5 polyethylene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

† N.D., none detected, limit of detection ca. 0·5 μg

CONCLUSION

We conclude from these results that nickel is indeed released from stainless steel prostheses by the action of sweat, blood and PSS. From the limited study in animals it appears that the solubilized nickel is localized in the tissue near the implant, but a longer-term, more comprehensive study is required to confirm this finding.

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Growth inhibition in normal mammalian melanocytes *in vitro*

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SUMMARY

Studies on primary cultures of normal adult melanocytes from the ear skin of a black strain of guinea-pigs are reported. Melanocytes have a low proliferation rate and tend to form cohesive groups on a glass surface. The cells undergo division while maintaining close contacts with each other whereas DNA synthesis is rare in melanocytes with multiple contacts.

Quantitative data on the distribution of epidermal melanocytes in the guinea-pig and in man have shown that the melanocyte density is constant within narrow limits depending on the epidermal architecture (Billingham & Medawar, 1953; Szabo, 1967). The population density of melanocytes is much less than that of the epidermal cells which they pigment, an observation which has led to the proposal of a specific structural relationship between melanocytes and keratinocytes (Fitzpatrick & Breathnach, 1963). In terms of mutual interactions between melanocytes and Malpighian cells a pattern of melanocyte distribution based on the disposition of individual zones of pigmentation (epidermal melanocyte units) has attractive features. The spacing of the pigment cells might be determined by positioning influences during migration or by the extrinsic influences on migration or melanization exerted by keratinocytes. However, studies *in vitro* (Pruniéras, Leung & Colson, 1964; Cruickshank & Harcourt 1964; Cohen & Szabo, 1968) do not demonstrate any numerically unique melanocyte–keratinocyte relationship. In this paper evidence is presented which suggests that the spatial distribution of melanocytes is the result of an extreme form of density-dependent growth inhibition.

METHODS

Culture of cells

The experiments were carried out on primary cultures of adult guinea-pig melanocytes. An inbred strain of black animals was used and fragments of ear skin of about 1 mm^2 area were removed freehand with a razor blade from animals under halothane anaesthesia. These pieces of skin were incubated for 30 min at 37°C in 0·4% trypsin in physiological saline. The dermis was removed with fine forceps and the epidermis washed twice with cold sterile saline. The melanocytes and basal keratinocytes were then picked up on flame-sterilized cover glasses by allowing the glass to rest lightly on the exposed basal layer of the skin fragment. The glass was then transferred to a culture chamber of the type described by Cruickshank *et al.* (1959). In the initial experiments Perspex chambers with a platform area of 3·2 cm^2 and a volume of 0·12 ml were used. These were later replaced by disposable chambers purchased from Sterilin Ltd., Richmond, England, which have a platform area of 2·05 cm^2 and a volume of 0·15 ml. The coverslips were sealed on to the chambers with bees-wax and culture medium introduced with a sterile syringe. Medium consisted of Eagle's basal medium with Hank's salts containing 5% fetal bovine serum, 0·5% chick embryo extract and penicillin and streptomycin added to give a final concentration of 1000 units per cent of each. These materials were obtained from Flow Laboratories Ltd, Irvine, Ayrshire, Scotland. Cultures were incubated overnight at 37°C with the glass downwards and the chambers then inverted according to the technique of Pruniéras (Pruniéras *et al.*, 1964). The medium was exchanged after 24 h for new medium which had been gassed with 95% oxygen and 5% carbon dioxide. Because of the greater resistance of melanocytes to high oxygen tensions this treatment enables pure melanocyte cultures to be obtained (Riley, 1970a). The cultures took about 48 h to become established. Counts of mitotic cells showed a steady decline in the number of divisions of the melanocyte population as the culture aged; the average total mitoses falling to about a quarter of their original value after 30 days in culture. Mixed cultures of melanocytes and keratinocytes prepared in the same way but omitting the hyperoxygenated medium showed no evidence of increased mitosis by the pigment cells and on occasions when keratinocytes grew well the littoral melanocytes showed no mitotic activity at all. Pigment transfers took place both between melanocytes and keratinocytes and between melanocytes giving rise to segregated packets of pigment granules in the acceptor cells. Unless otherwise stated the studies referred to in the text were made on cultures between 4 and 10 days after seeding. The metabolic status of the cells was established by cytochemical and radio-autographic techniques.

(1) *Cytochemical techniques*

Two enzymes were investigated at various times in cells fixed for 5 min at 4°C with neutral 0·05 M barbital buffered formol-calcium. Tyrosinase activity was estimated by the dopa reaction; the method employed was as previously described (Riley, 1970b). Nucleoside polyphosphatase activity was demonstrated using ATP as substrate and the method was as described elsewhere (Riley, 1966). Intravital staining with methylene blue (0·1%) and with the fluorochrome acridine orange (0·1%) was also employed.

(2) *Radio-autographic techniques*

Incorporation studies were made using ^{3}H-thymidine (5 μCi/ml), ^{3}H-uridine (5 μCi/ml), ^{3}H-leucine (5 μCi/ml), ^{3}H-tyrosine and ^{3}H-dopa (3 μCi/ml) obtained from the Radiochemical Laboratories, Amersham, Buckinghamshire, England. The figures refer to the final concentration of the radioactive material in the medium. In the case of tritiated tyrosine additions to the medium, pulse lengths of 30 min and 12 h were used, in order to distinguish between uptake due to tyrosine activity and protein synthesis. The labelling periods for leucine, dopa and uridine were 2 h. The labelling regime for thymidine involved a pulse time of 6 h. Fixation of material for autoradiography was in 4% glutaraldehyde in 0·1 M phosphate buffer at pH 7·4 for a minimum of 2 h at 4°C, after which the cover glasses were removed from the chambers and the cultures washed with distilled water, processed through graded ethanols to absolute ethanol and placed in a 37°C incubator to dry. The autoradiographic technique was modified from that described by Kopriwa & Leblond (1962) for use with coverslips. The coverslips were marked on the reverse surface and suspended with clips, or held with forceps, over a beaker while warmed emulsion was carefully layered on to the cell-covered side. By tilting the coverslip the excess emulsion was allowedt o run off into the beaker and drying was completed with a cold air fan. The emulsion used was Ilford K2 Nuclear emulsion in gel form and the exposure time was 1 week at 4°C. Cells were counterstained with Giemsa 'R 66' (G.T.Gurr & Co. Ltd.) and the material dehydrated, cleared in xylol and mounted in DPX. Some experiments using thymidine labelling were made in conjunction with colchicine treatment. Colchicine was used in a final concentration of 10^{-7} M and the cells examined 12 h after exposure to this agent.

(3) *Electron microscopy*

Material for electron microscopy was fixed by infusing the culture chambers with glutaraldehyde, the fixation procedure being the same as used for autoradiography. The coverslips were then removed and the material washed, dehydrated and embedded in Araldite. The glass was removed by floating the block on a bath of hydrofluoric acid and the exposed portion embedded in Araldite. Sections were cut on a Porter-Blum Ultramicrotome using diamond knives, stained with lead citrate and uranyl acetate and examined in a Siemens Elmiskop 1 electron microscope.

(4) *Time lapse cinematography*

Cultures were examined by time lapse cinematography using a Vinten mark II camera mounted on a phase contrast microscope in a constant temperature enclosure. Intermittent green light illumination of low intensity was used. The lapse times were varied between 10 and 60 s. Examination of cells in a chosen field was for a minimum of 12 h within 24 h of a medium change. Analyses were made by examination of formation and loss of contact in terms of the frame numbers and the times calculated.

(5) *Cell counts*

The distribution of cells in the cultures was examined and measurements made of the cell size, numbers of contacts between cells and the length of contacts using an eyepiece graticule. The distribution of the labelling in groups of melanocytes of different sizes was recorded.

The standard statistical procedures were used. In order to eliminate bias in the large groups where only small numbers of observations were available the reciprocal of twice the number of cells counted was substituted for zero values. Also in calculating the variance of the sample for different group sizes a weighting factor was introduced to take into account the number of observations in each group.

RESULTS

Attachment and growth

Observation of the cells during attachment showed that the rates of attachment of keratinocytes and melanocytes were similar but it was evident that the spreading of melanocytes on glass occurred more rapidly. Attachment was completed for most cells after 6–10 h. Normally, the chambers were inverted after 16 h and a substantial number of keratinocytes remained on the cover glasses at this stage but subsequently rounded up and fell off when oxygen-rich medium was introduced. During the next 24 h, the melanocytes tended to aggregate and several successive divisions occurred in such aggregates giving rise frequently to multinucleate cell masses which had the appearance of syncytia. Stained preparations of cells fixed at this stage showed the presence of membranes between nuclei. At a later stage (48 h after seeding) the cells separated and spread themselves more widely on the glass.

Morphology

At this stage the cells took up their characteristic morphology (Fig. 1). The cells were rather angular in outline and measured on average 2100 μ^2 in area. The measurements summarized in Table 1 show that there was no significant difference in the cell area of labelled and unlabelled cells or of cells in different sized groups.

The nuclei characteristically showed between three and five nucleoli and were rather sparsely overlayed by pigment granules which tended to aggregate peripherally round the nucleus, often with

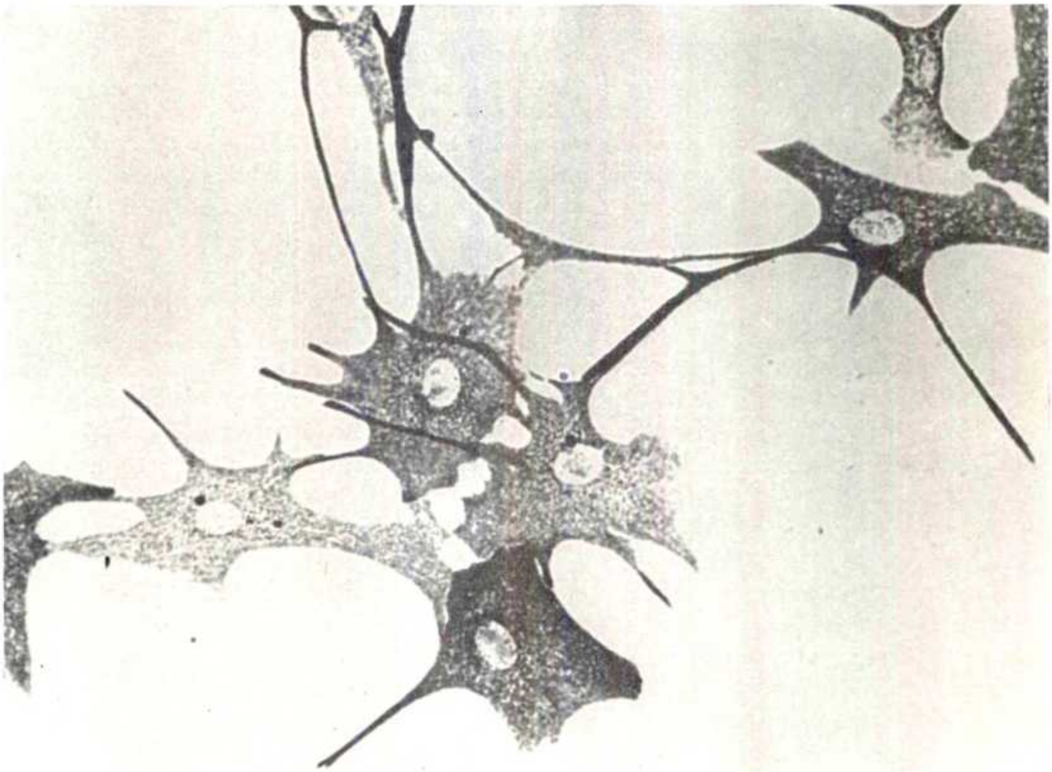

FIGURE 1. Normal guinea-pig melanocytes in 5-day-old primary cultures showing multiple dendritic contacts. In general dendrites pass over the dorsal surface of the cells. Note the extensive lateral abutments between cells. The cell in the centre of the picture displays a thin lamellar extension towards the cell on the right hand side (Phase contrast × 1250).

TABLE 1. Cell area and lengths of contact with neighbouring cells

Group size	Thymidine labelled cells in group		No labelled cells in group	
	Area (μ^2)	Contact length (μ)	Area (μ^2)	Contact length (μ)
1	1663 ± 816	—	2287 ± 638	—
2	2092 ± 265	23·77 ± 15·28	1597 ± 1000	28·02 ± 20·51
3	2217 ± 641	26·24 ± 13·77	1730 ± 665	27·27 ± 13·31
4	2184 ± 391	16·84 ± 10·69	2244 ± 637	20·82 ± 16·35
5	2370 ± 605	10·18 ± 6·22	1942 ± 760	15·56 ± 17·75
> 5	2218 ± 480	24·62 ± 11·93	2056 ± 601	30·83 ± 31·70

Figures give mean ± s.d.

a bias to one pole. The dense granular portions of cytoplasm were surrounded by a thin filmy zone (Fig. 2) which in many cells showed evidence of axes of linear stretching. Occasionally, pigment granules were seen lined up along these axes. After 96 h in culture it was not unusual to see cells in which the degree of pigmentation was greatly reduced and in some cases absent altogether. This was ascribed

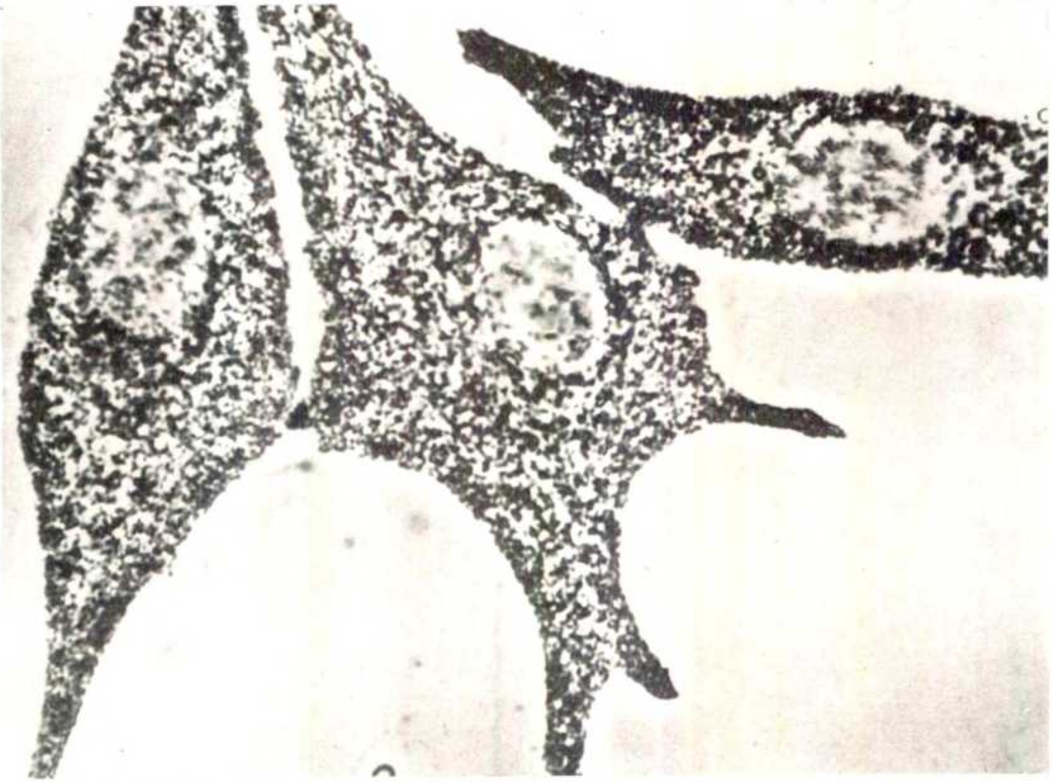

FIGURE 2. Normal guinea-pig melanocytes 4 days after seeding showing the lamellar contact interfaces formed between the cells (× 1750).

TABLE 2. Autoradiography—grain counts per cell

Label	Pulse duration (h)	Melanocytes	Keratinocytes
^{3}H-Tyrosine	0·5	21·08 ± 9·98	4·68 ± 1·28
	12	73·42 ± 50·60	110·45 ± 51·19
^{3}H-Uridine	2	7·93 ± 3·15	11·90 ± 8·66
^{3}H-Leucine	2	43·31 ± 25·50	142·06 ± 60·01
^{3}H-Dopa	2	29·61 ± 11·14	5·36 ± 2·23*
	2†	55·58 ± 33·20	20·48 ± 10·04

The figures refer to the mean grain numbers per cell ± the standard deviation. All counts were made on 25 arbitrarily chosen cells.

* Background = 5·36 per equivalent melanocyte area.

† Fixed material.

to pigment dilution due to cytoplasmic proliferation in the concurrent absence of further pigment synthesis. The dopa reaction was absent or diminished in these cells. Both the heavily pigmented and unpigmented cells stained similarly with methylene blue and acridine orange. The cells did not stain for nucleoside polyphosphatase. Unpigmented cells were not observed in early cultures.

RNA synthesis

Autoradiographic results showed that RNA synthesis took place in the cultured cells (see Table 2). Comparisons made in mixed keratinocyte and melanocyte cultures showed that the tritiated uridine

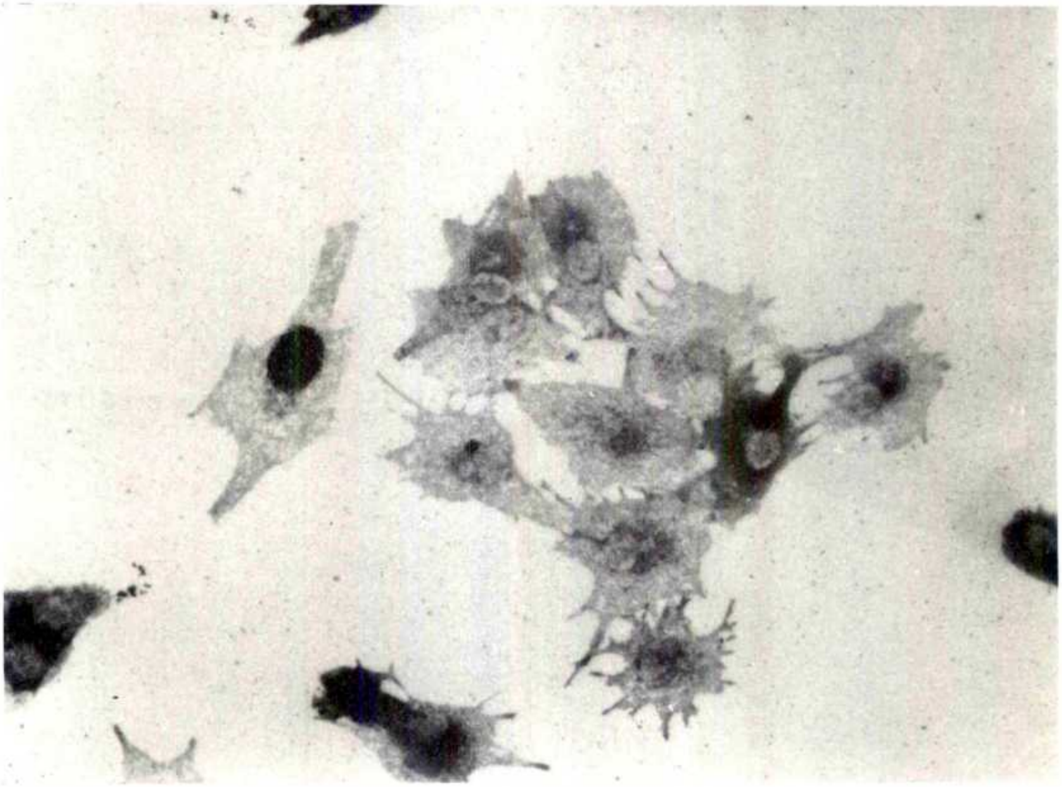

FIGURE 3. Autoradiograph of melanocytes labelled with ^{3}H-TdR. Note that the heavily labelled cell is isolated. The cells in contact are unlabelled (× 400).

TABLE 3. Distribution of ^{3}H-TdR labelling in groups of different size

No. of cells in contact	No. of cells	(%) Labelled cells ± .d.
1	2364	18·27 ± 2·90
2	1578	10·89 ± 2·72
3	1188	3·17 ± 1·17
4	1140	0·71 ± 0·37
5 <	1213	0·93 ± 0·35

uptake in pigment cell nuclei following a short pulse was approximately 50% of that found in keratinocytes.

Protein Synthesis

Protein synthesis was considerably diminished in melanocytes in comparison with keratinocytes,

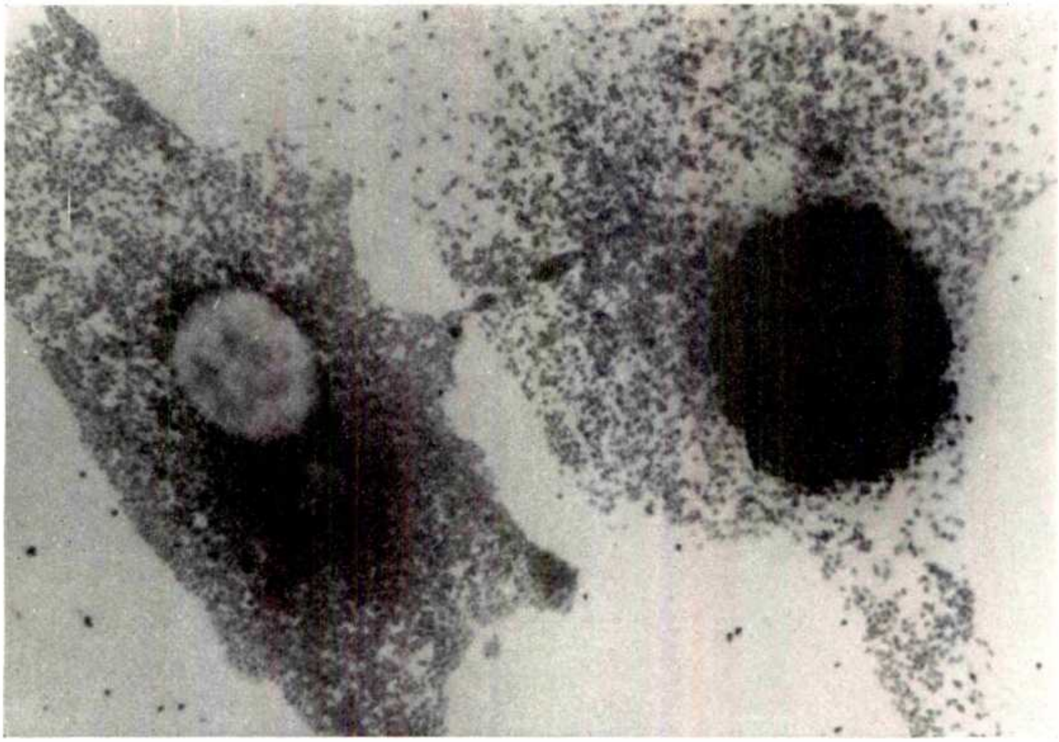

FIGURE 4. Autoradiograph of ^{3}H-TdR labelled melanocytes. One cell is heavily labelled with an enlarged nucleus and is in contact with another cell which has failed to take up the label illustrating non-synchrony of DNA synthesis in contacting cells (× 1600).

TABLE 4. Influence of period in culture on the mitotic index of melanocytes

Time in culture (days)	Mean mitoses per 10^3 cells ($\pm$ S.E.M.)
1	8.2 ± 1.2
2	4.1 ± 1.7
3	4.5 ± 0.6
4	5.7 ± 1.7
5	4.8 ± 0.2
6	5.0 ± 0.8
7	3.9 ± 1.2
8	2.6 ± 0.9
9	3.2 ± 2.1
12	2.7 ± 0.7
30	1.8 ± 0.6

being about 10% as judged by the number of grains per cell when tritiated leucine and tritiated tyrosine were employed as indicators of the rate of protein synthesis (Table 2).

DNA Synthesis

The cultures were examined between 2 and 10 days after seeding. In these the labelling index averaged about 1·3%. However, the distribution of labelling was not uniform in the cultures and a reciprocal relationship between the incorporation of tritiated thymidine and the number of cell contacts was evident (Figs. 3 and 4; Table 3). The cells showed no synchrony of labelling within groups.

Mitoses

Counts made of colchicine-arrested metaphases showed that there was a gradual fall in mitotic activity in the cultures despite medium changes at 48 h intervals (Table 4). An analysis on the basis of group sizes did not show the same distribution as the results for DNA synthesis (Table 5).

TABLE 5. Distribution of colchicine arrested metaphases in groups of different sizes

No. of cells in contact	No. of cells	(%) Metaphases ± S.D.
1	364	6·04 ± 3·41
2	578	8·82 ± 2·85
3	188	6·38 ± 2·74
4	140	12·14 ± 4·33
>5	213	8 45 ± 1·96

Motility

The melanocytes moved in a manner reminiscent of fibroblasts, by extension of thin veils of cytoplasm with a leading ruffling edge. The granule-containing cytoplasm with the nucleus frequently formed a crescent-shaped trailing portion. Membrane ruffles were also seen in zones where pinocytosis was taking place. Pinocytosis was stimulated by medium changes as observed both directly by time-lapse cinematography and also indirectly by the uptake of acrydine orange into vesicles in the melanocyte cytoplasm. The time-lapse studies showed that there was very considerable movement of melanocyte cytoplasm but very little real movement of the cells on the glass. The range found for single cells with no contact with other cells was 0·42–7·8 μ^2/h for fifteen samples averaged over 8 h. Membrane movements appeared to be random in direction. Exploratory movements involving considerable cytoplasmic expansions at the margins took place often with the simultaneous extension of dendrites. The dendrites frequently did not make contact with the glass substrate and were most often extended from regions of the cell which were relatively dense and stable.

Dendrites

The dendritic extensions have a characteristic structure consisting of a cylindrical process of cytoplasm containing melanosomes in various stages of melanization and mitochondria. The distal ends of these processes are rounded and a hair-like process was frequently observed at the tip which extended as much as 5 μ from the pigmented end of the dendrite. This filiform process was frequently curved and in some instances could be seen to have a movement independent of the direction of extension for the dendrite. The dendrites themselves showed a tendency to extend linearly from the margin of the cell but occasionally showed abrupt changes in direction. Forking also took place. Occasionally several branches were formed on the same original process but always by a bifurcation. Broadening of the tips was sometimes seen when direct contact with another cell was made and occasionally wedge-shaped abutments were formed to make contact with adjacent cells.

Contacts

(a) *Contacts between melanocytes and keratinocytes.* It was observed that when a melanocyte process approached a keratinocyte the keratinocyte frequently displayed some membrane activity in relation to the advancing tip, possibly initiated by contact with the terminal microfilament. Occasionally, terminal portions of the dendrite were engulfed by the keratinocyte. This phenomenon has been previously described (Cruickshank & Harcourt, 1964; Cohen & Szabo, 1968). In this study it was frequently observed that melanocytes withdrew from contact with keratinocytes.

(b) *Contacts between melanocytes.* Melanocytes showed a marked tendency to make contacts with one another. These were frequently made while the cell bodies remained separated by some considerable distance and involved the approach of two or more dendrites. These would make contact either by end to end anastomoses, when some expansion of the terminal portions was often seen, or by forming long contacts by lateral juxtaposition. The effect of this when several cells were in contact with one another through these branched processes was of a fine network.

Occasionally, the donation of pigment from one melanocyte to another was judged to have occurred because inclusions of the type seen in keratinocytes to which pigment had been transferred were seen. However, on no occasion was such a transfer between melanocytes actually observed.

Another form of contact between melanocytes which was observed formed in a different way by collision between rapidly moving cells. These cells tended to take up the 'crescent' appearance and moved in apparently random directions. When the ruffled membrane came into contact with another cell a tendency to move preferentially towards this cell was observed until a considerable length of juxtaposed membrane had formed. The manner in which this movement occurred was similar to reannealement between the membranous extensions of single cells which might suggest that the cells are unable to distinguish between their own membrane and that of another similar cell.

Extended regions of contact were found to form both between the granule-free margins and the thicker cytoplasmic zones. Occasionally, cells would overlap by about half their surface area. Similarly, contacts between cells and their own dendrites, and between dendrites from different cells were apparently extremely close with dendrites running parallel, closely applied to one another. Branching dendrites were observed frequently and self-contact with dendrites was observed on several occasions. The zone of movement of individual melanocytes was usually limited to about twenty cell diameters and stable positional relationships were formed when several cells were in contact. The contact distances varied considerably and in the case of cultures where the initial growth rate was high the cell density approached 1000 cells/mm^2. In general, however, the size of cell groups was small; the distribution of group sizes is shown in Table 3. Time lapse observations showed many divisions occurring in the cell groups, which is in agreement with the data on the proportion of colchicine-arrested metaphases shown in Table 5. Frequently the dividing cells remained in contact through dendritic extensions with other cells in the group. Cells were observed to detach themselves from groups and re-attach either to the same group or to other cells in the vicinity. The contact frequency for isolated cells was less than one contact/600 min. For cells already in contact new contacts were observed to form at about 100–200 min intervals (mean value for seven cells, 139 min).

The average time of retention of unstable contacts was found to be 240 min in observations on seven cells. Stable contacts lasted throughout the observation period (40 h).

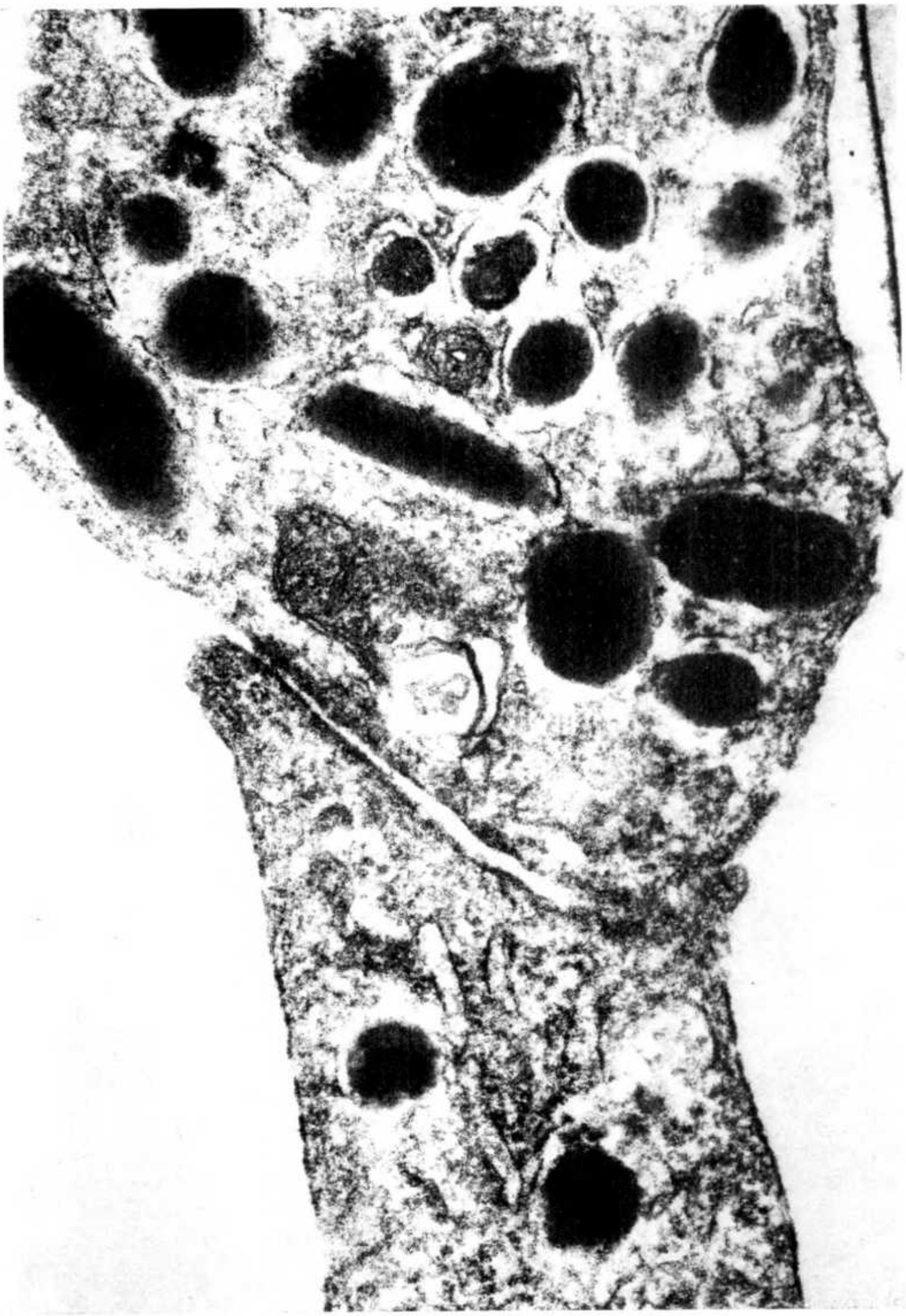

FIGURE 5. Electron micrograph of contact between melanocyte dendrites showing the narrow interstitial space (× 72,000).

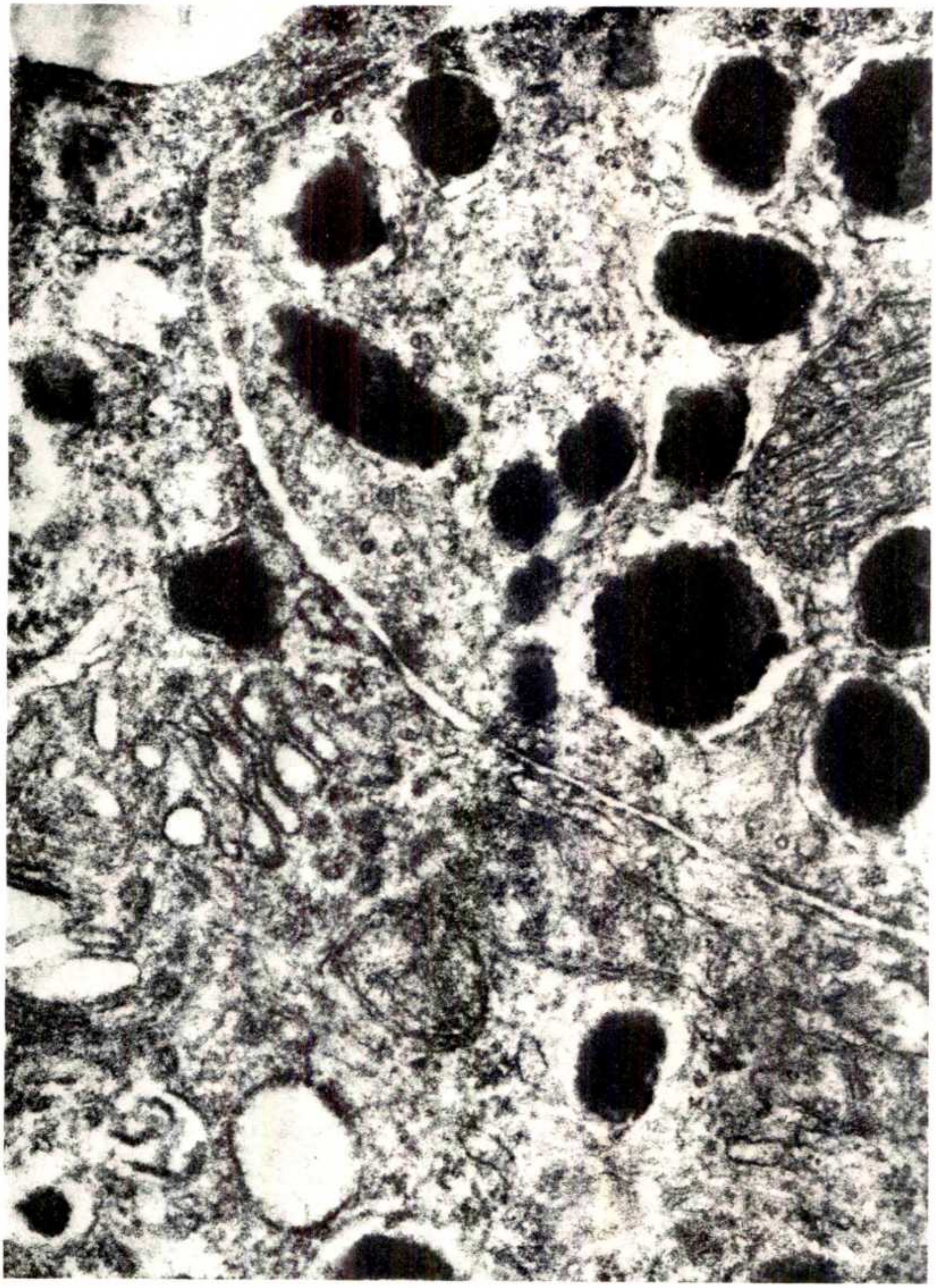

FIGURE 6. Electron micrograph of a region of contact between a cell and its own dendrite. The appearances of the space are similar to those shown in the previous figure. There is evidence of some condensation in the membrane (× 72,000).

The lengths of the stable contacts in groups of melanocytes are shown in Table 1. The results show that there is no significant difference between the contact lengths between groups cells in groups including and groups excluding cells labelled with tritiated thymidine.

The nature of the contact between the dendrites was somewhat non-specific. In some instances areas of condensation were observed in the space between the cells (Fig. 5). However, no structure resembling desmosomes was seen in any of the material examined by electron microscopy. The appearance of the junctional zones was similar in all cases, there being about a 10 nm separation between the cell membranes. No specific organelles associated with the contact regions were observed although. occasionally a region of increased electron density between the cell margins was observed (Fig. 6) On no occasion was continuity between the cytoplasm of one cell and another demonstrated, and analyses of time-lapse films failed to show any instance in which visible cytoplasmic components were exchanged.

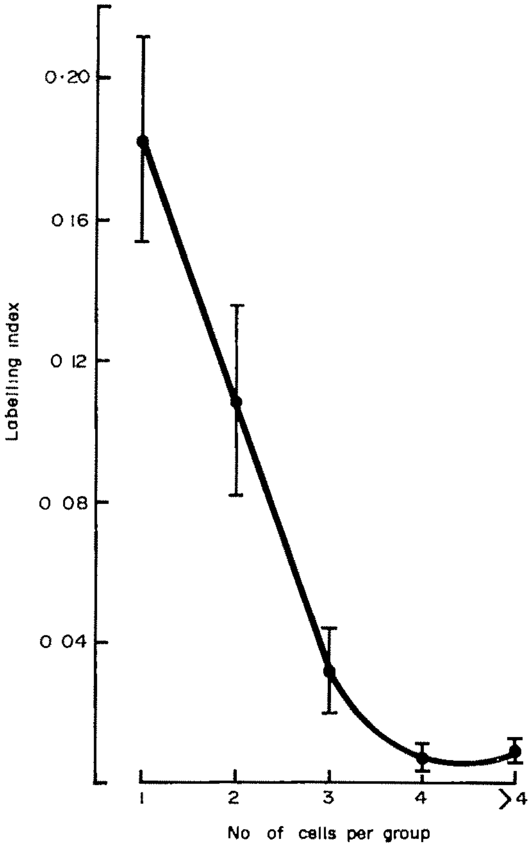

FIGURE 7. Graph showing the relationship between the labelling index and size of the groups of cells in contact. The vertical bars indicate the standard error of the means.

DISCUSSION

The evidence presented shows that melanocytes derived from adult mammals synthesize RNA and protein, elaborate tyrosinase, form pigment and are capable of division. The reduction of histochemically demonstrable tyrosinase activity might have been expected to reduce the degree of pigmentation of the material over a short period. However, in cultures containing only melanocytes, where the opportunity for the distribution of melanin was limited, the majority of cells remained pigmented for long periods. It was noticeable that cells which underwent successive divisions became less pigmented by virtue of the dilution of melanin granules.

Growth Regulation

The rate of growth of the melanocyte population studied was clearly limited by cell contact with other melanocytes (Fig. 7). The degree of inhibition produced by relatively few contacts was very marked and the numbers of contacts that the cells were able to make was greatly increased by the lengths to which the dendrites could be extended. Thus, contacts with cells up to 10 cell diameters away were not infrequently seen.

The most probable explanation for the inhibition of DNA synthesis observed in this study is that it is mediated by some agent passing between the cells, possibly by a substance diffusing across the parasynaptic gap separating the cells. Alternatively a direct interaction at the surface may cause some cellular change which inhibits the synthesis of DNA. The sparsity of the culture, the lack of spatial constraint, and the relative smallness of the regions of the cells which are in contact make it improbable that inhibition of cell growth in this system is the result of relative deprivation of nutrients either through changes in cell shape with consequent reduction in available exchange surface or local changes in the medium. The absence of synchronous thymidine labelling in groups of contacting cells may indicate that metabolic coupling is unlikely to contribute significantly to the inhibition of DNA replication.

By contrast with DNA synthesis, mitosis was not found to be influenced by the number of contacts between cells. These observations would appear to favour the hypothesis that melanocytes mutually inhibit their proliferation in the circumstances imposed by monolayer culture by inhibition of DNA synthesis and suggest that the mechanism involves an external influence which operates directly or indirectly on the surface of the cell, mediated through stable intercellular contacts which are able to form between dendrites.

If these observations in any way reflect the growth control mechanisms operating *in vivo* they may go some way towards explaining the stability of the relatively sparse population density of melanocytes.

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Primary cell culture for biochemical studies of human keratinocytes

A METHOD FOR PRODUCTION OF VERY LARGE NUMBERS OF CELLS WITHOUT THE NECESSITY OF SUBCULTURING TECHNIQUES

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SUMMARY

Starting with large numbers of small split-thickness explants of human skin, it is possible to grow ample numbers of keratinocytes *in vitro* as primary cell cultures to permit biochemical studies without the need of subculturing techniques.

Primary cell (outgrowth) cultures of human keratinocytes, obtained from small split-thickness pieces of skin, have been used by several investigators studying the basic biological properties of these cells. Past studies have defined the ability of keratinocytes to form a histologically organized keratinizing epithelium (Flaxman, Lutzner & Van Scott, 1967; Karasek, 1966a; Friedman-Kien *et al.*, 1966; Pinkus, 1932; Pruniéras, Gaté & Bonjean, 1958). They have also been useful in analysing cell communication (Cavoto & Flaxman, 1972), the cell cycle (Chopra & Flaxman, 1972, 1974; Flaxman & Chopra, 1972), response to pharmacological agents (Chopra & Flaxman, 1973; Chopra, Yu & Flaxman, 1972; Chopra, Cherkas & Flaxman, 1974; Harper & Flaxman, 1974; Harper, Flaxman & Chopra, 1974a, b; Karasek, 1970, 1971) and the effect of chemical carcinogens (Dietz & Flaxman, 1971). However, it has been difficult to subculture the keratinocytes progressively to yield large numbers of cells for biochemical work. We here report a slight modification of existing techniques which permits the growth of large numbers of human keratinocytes without the need for subculturing.

MATERIALS AND METHODS

The method of obtaining primary outgrowths of keratinocytes, free from fibroblast contamination, has been described in detail in previous publications (Friedman-Kien *et al.*, 1966; Karasek, 1966a; Flaxman *et al.*, 1967). Generally, a small number of explants (1–5), each 1–2 mm across, have been placed on a coverslip in a 35 mm diameter Petri dish. The explants were held in place by a plasma clot comprised of chick embryo extract and plasma, and immersed in the appropriate culture fluid such as Eagle's minimal essential medium. In the present study, large pieces of abdominal skin (about 2 × 10 cm) obtained during surgery were used as the source of material. Thirty split-thickness explants of equal size were spaced evenly on the bottom of Petri dishes 60 mm in diameter. A thin film of chick plasma was first spread over the bottom of each dish. The explants were arranged in place and chick embryo extract added and gently mixed with the plasma. The ratio of plasma to embryo extract was 3:1 (v/v). The same total volume of clotting mixture was used for each dish (0·15 ml per 60 mm dish). The entire assembly was allowed to clot for approximately 1 h before culture fluid was added. Up to fifteen dishes could be made from each strip of surgical skin. In the hands of experienced personnel, about 3 h were required to complete this task.

Using 3-week-old cultures, cell extracts were made according to a modification of the method of Schmidt & Thannhauser (1945). The culture fluid was removed by aspiration and the cells were washed twice with 3·0 ml of ice-cold Gey's balanced salt solution. Explants were then removed with fine forceps and discarded and the outgrowths scraped loose with a rubber policeman and placed in a centrifuge tube. 1·5 ml of ice-cold 5% trichloracetic acid (TCA) was added, followed by centrifugation at 2,000 *g* for 10 min at 4°C. After discarding the supernatant fraction, the pellet was resuspended in 1·5 ml of ice-cold 95% ethanol, centrifuged again and the supernatant fraction discarded. The pellet was then suspended in 1·5 ml of 5% TCA, heated for 20 min at 90°C and centrifuged. The supernatant fraction (I) was retained for DNA and RNA determinations. The remainder of the pellet was suspended in 2·0 ml of 0·1 N NaOH for 5 min and then centrifuged. The supernatant fraction (II) was retained for protein analysis. Additionally, in some instances washed cells on the bottom of the dish were treated directly with 1% sodium dodecyl sulphate for 30 min at 37°C, during which the cells were lysed. Following centrifugation, the resulting supernatant fraction was analysed for protein.

Tritiated thymidine (0·08 mCi per dish; sp. act. 20 Ci/mM) was added to several dishes at the same time and cultures terminated at 1 h intervals. Following extraction of DNA according to the above method, counts were made in an Intertechnique SL 30 liquid scintillation spectrometer.

RESULTS

Each explant gives rise to an outgrowing ring of keratinocytes which enlarges due to active mitosis and outward cell migration. After about 3 weeks in culture, the keratinocyte sheets become confluent (Fig. 1) so that the entire bottom of each dish is covered by the epithelial cells. If the procedure is carried out with care, it is possible to avoid fibroblast growth. Fibroblast contamination, if present, is readily detectable by the distinctive growth pattern, and any contaminated dishes discarded.

The area of a 60 mm diameter Petri dish is 2826 mm^2. In the dishes with thirty starting explants, total explant area (or epidermal surface area) was measured directly and found to be approximately 56 mm^2. Thus, after 3 weeks in culture, at the point of confluency, the original keratinocytes had proliferated to cover an area about fifty times greater than that covered by the starting material. This represented 4·75–5 × 10^6 cells per dish, as determined by a direct counting method described elsewhere (Chopra *et al.*, 1972).

A volume of 0·8 ml of supernatant fraction (I) was used to estimate DNA employing the diphenylamine reaction (Burton, 1968). Total DNA in a 60 mm dish was about 36 μg. Starting with the same number of explants, the total amount of DNA from dish to dish varied by only 10%. Knowing the quantity of cells and DNA per dish, it was calculated that each keratinocyte contains about 7×10^{-6} μg of DNA, a figure in agreement with earlier *in vivo* determinations made by microspectrophotometry (Leuchtenberger & Leuchtenberger, 1954). The further usefulness of this system in studying

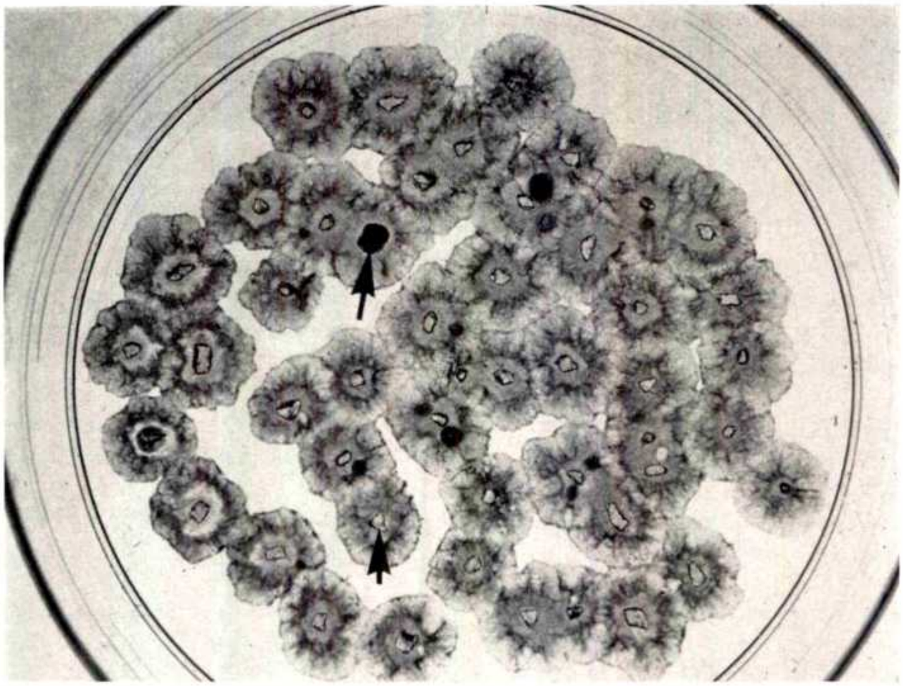

FIGURE 1. Light photomicrograph of Petri dish containing multiple explants (arrows) of split-thickness human abdominal skin. More or less circular epithelial outgrowths surround each explant. In this 2-week-old culture, outgrowths are already confluent in many places. After an additional week, entire bottom of dish will be covered. Formalin fixation. (H & E, × 1·3).

DNA synthesis was shown by the ability to demonstrate a linear rate of incorporation of ^{3}H-thymidine into DNA. A typical curve is shown in Fig. 2.

A supernatant fraction (I) of 0·2 ml was used as the source of RNA which was estimated by the orcinol test according to Schneider (1957). The total amount of RNA in a 60 mm dish was about 18·0 μg.

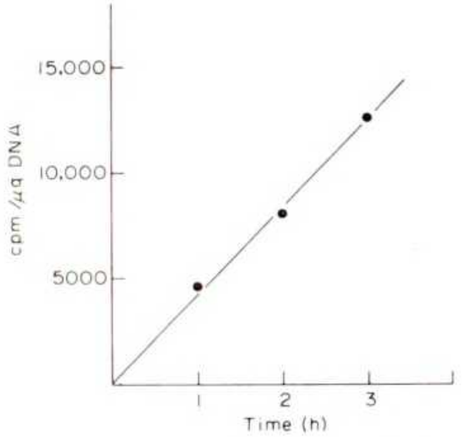

FIGURE 2. Graph showing linear incorporation with time of ^{3}H-thymidine into DNA.

A supernatant fraction (II) of 0·1 ml was used as the source of hydrolysed protein which was estimated by the procedure of Lowry *et al.* (1951). The total amount of soluble protein in a 60 mm dish was about 880 μg. However, by lysing the intact cells with 1% SDS, up to 2·5 mg of protein could be recovered from each dish, indicating the presence of a normally insoluble protein, probably keratin (Karasek, 1966b, 1970).

DISCUSSION

Starting with amounts of skin often available from routine surgical procedures (10 × 2 cm), it is possible to obtain viable, proliferating human epidermal keratinocytes as primary cell cultures in numbers sufficient to permit biochemical studies. Thus, it is not necessary to build up the cell population through repeated subculturing. The difficulty of subculturing human keratinocytes, in contrast to those of the guinea-pig, is well-known to investigators in this field, but the reasons for this difficulty are poorly understood (see review by Flaxman, 1974). A possible drawback of the present method is that a fresh supply of skin for new primary cultures must be continually available. Nevertheless, it is hoped that the ability to grow many millions of keratinocytes in primary cell culture will encourage investigators to greater use of the *in vitro* system in study of the biochemistry of the human epidermis.

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The junctional zone beneath migrating epidermis*

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SUMMARY

The changes that take place in skin explants *in vitro* at the interface between migrating epidermal cells and dermis have been examined by light and electron microscopy and by immunofluorescent antibody techniques. In the first 24 h epidermal cell migration occurred in the absence of any of the usual components of this junctional zone. After 48 h of incubation antigenic determinants of the basal zone were detected and basal lamina was demonstrated ultrastructurally. It is proposed that migrating epidermal cells do not need a complex and specialized junctional zone and that these cells are themselves responsible for the elaboration of new basal lamina.

Epidermal cell migration (ECM) takes place within a few hours of wounding and is the first stage of orderly re-epithelialization. Martinez has shown, for example, that ECM takes place from the margins of wounds in the buccal mucosa in as short a time as 4 hours (Martinez, 1971). Migrating epidermal cells are also capable of penetrating fibrin clot and intact connective tissue (Winter, 1971). Clearly the usual relationship between the dermis and epidermis beneath migrating epidermal cells is not preserved. Despite the absence of a normal dermo-epidermal (D/E) junction this area is rapidly reconstituted. Because of our interest in re-epithelialization in the wound healing sequence we have studied the D/E junction beneath the epidermal outgrowths that arise from the cut edges of skin explants *in vitro* (Medawar, 1948; Sarkany, Grice & Caron, 1965; Cruickshank, 1965; Marks, Bhogal & Dawber, 1972). We believe that these outgrowths arise from active ECM rather than from mitotic activity and regard them as analogous to the epithelial tongues seen in the initial stages of epidermal wound healing (Nishikawa & Marks, 1973).

The junctional zone between dermis and epidermis is complex and consists of the basal lamina and modified components of connective tissue. It also possesses antigenic components (whose anatomical counterparts are unknown) which are detectable by immunofluorescent staining using an antibody found circulating in the blood of patients with bullous pemphigoid.

In this study we have compared the junctional zones of migrating and non-migrating epidermis of skin explants *in vitro* by; (1) examining the basal region ultra-structurally; (2) examining the connective tissue of this zone by light microscopy; (3) using immunofluorescence methods to detect the presence of the basal region's antigenic components.

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MATERIALS AND METHODS

Human skin was obtained with a Castroviejo Keratotome set at 0·4 mm from the lateral aspect of the thighs of young adult male volunteers who had given their informed consent. The thin sheets of skin were divided into rectangular explants of approximately 60–70 mm^2. The skin explants were floated epidermis upwards on Eagle's minimal essential medium containing 10% pooled normal human serum. A volume of 5 ml of the medium and two or three of the explants were placed in small plastic petri dishes (sterilin). Incubations were performed at a temperature of 37°C in an atmosphere of 95% air and 5% carbon dioxide and were for periods of 1–5 days.

Altogether seventy-six explants were incubated and evaluated in these experiments. After incubation they were treated as follows:

(a) Thirty were fixed in 10% buffered formalin and were processed histologically by the usual methods. Sections from each specimen were stained by haematoxylin and eosin, periodic acid Schiff reagent and for reticulin. These sections were examined by light microscopy.

(b) Thirty-seven explants were 'snap' frozen in liquid nitrogen and were sectioned on a Bright's cryostat set at 6μ. Sections from each explant were incubated with serum from a single patient with proven pemphigoid and other sections were incubated with serum from another patient with proven pemphigus. The titres of these sera were in excess of 1:640. The incubations were for periods of 30 min and were performed in moist chambers. After this initial incubation and washing in phosphate buffered saline the sections were incubated for an additional 30 min with sheep anti-human IgG labelled with fluorescein isothiocyanate (Wellcome Reagents) with an F/P ratio of 3·6 M and diluted 1:20 with normal saline. The sections were then washed with phosphate buffered saline and were examined in a Leitz SM Fluorescence microscope. Positive and negative controls were run with each batch and 'blocking' experiments were performed by pre-incubating with anti-human sera not conjugated with fluorescein.

(c) Nine specimens were fixed in 2·5% glutaraldehyde for 2½ h and were post-fixed in osmium tetroxide prior to dehydration in graded alcohols and embedding in araldite. The araldite-embedded tissue was sectioned on a Leitz ultramicrotome and silver-grey and gold sections were examined in an A.E.I. 6B electron microscope.

RESULTS

Light microscopy

Detailed inspection of the cultured explants made it obvious that, at least in fixed preparations, there was not a firm union between migrating epidermal cells and the dermis over which they had migrated. Explants maintained for more than 3 days appeared to develop a firmer relationship between dermis and epidermis. A PAS positive 'band' was observed beneath the epidermis of the body of the explant but was absent beneath the migrating epidermis. The PAS positive band ended abruptly at the start of the area of migration (Fig. 1). In two 72 h specimens there appeared to be faint areas of PAS positivity subepidermally just beyond the start of the epiboly. The specimens stained for reticulin yielded similar findings although the areas of reactivity were less easily defined.

Immunofluorescence Studies. The dermo-epidermal junction of the main part of the explants demonstrated the usual band of fluorescence after incubation with pemphigoid serum in all the specimens tested indicating that the antigenic components of the basal region were retained after culture for periods of up to 120 h. The fluorescent band did not extend into the subepidermal zone of the migrating portion of the explant in the majority of specimens (Fig. 2). However, in five of the thirty-three specimens there was a fragmented band of fluorescence (Fig. 3). Two of these five specimens had been

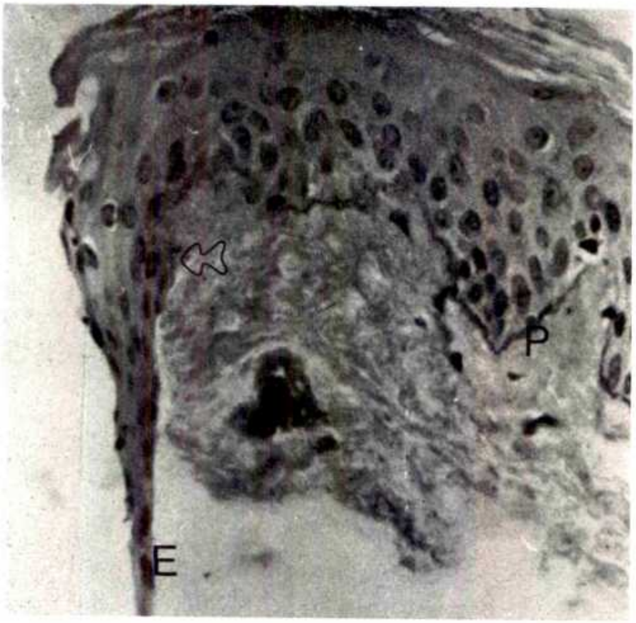

FIGURE 1. After 24 h of incubation there is an epithelial outgrowth (E) which is not firmly adherent to the dermis. A PAS positive band (P) is not present at the junctional zone of the migratory epidermis (↑) (Periodic acid Schiff, × 180).

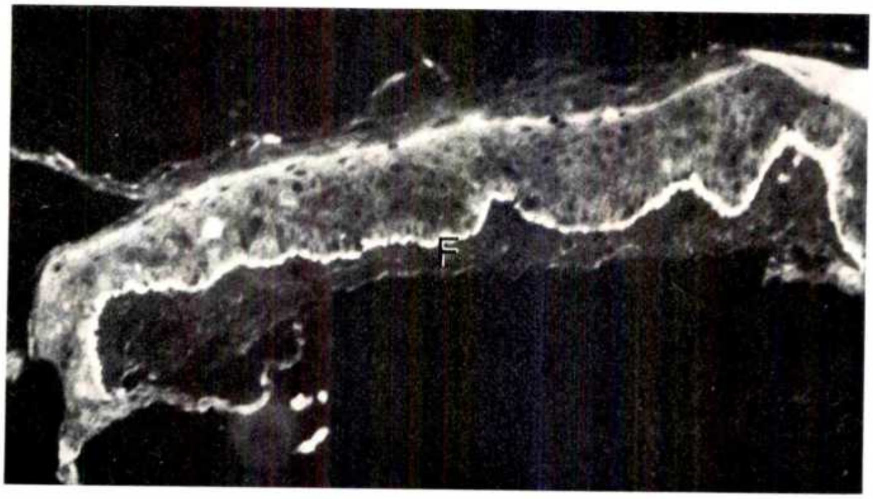

FIGURE 2. Immunofluorescence after 24 h of skin explant culture and subsequent incubation with pemphigoid serum; stained with FITC labelled anti-human IgG. A fluorescent band can be seen at the dermo-epidermal junction (F) which stops abruptly at the start of the epithelial outgrowth (× 135).

incubated for 48 h and the amount of fluorescent material in these was very small. The other three specimens had been maintained in culture for 72, 96 and 120 h respectively and although the fluorescent area was fragmented it was obviously a linear band-like zone. The numbers showing a positive pemphigoid type fluorescent band and the site of these bands is shown in Table 1.

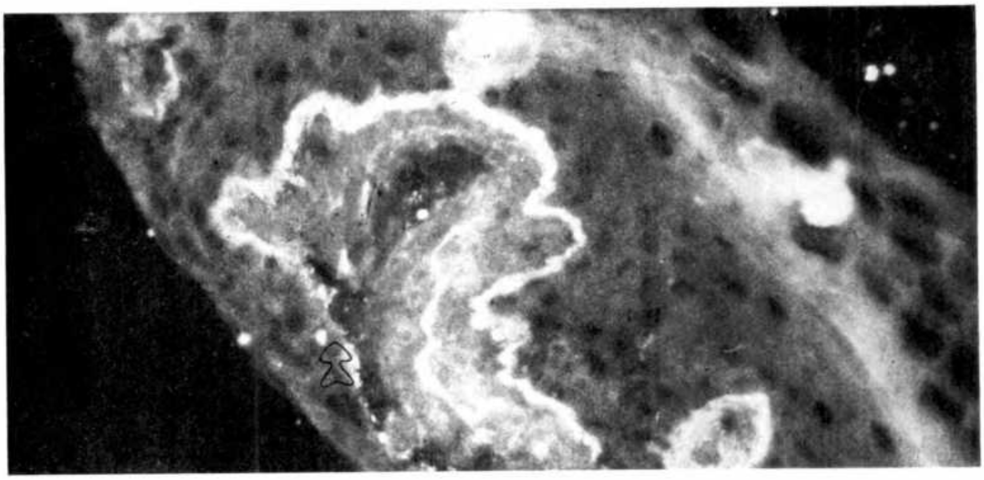

FIGURE 3. Immunofluorescence after 48 h of skin explant and subsequent incubation with pemphigoid serum; stained with FITC labelled anti-human IgG. A fluorescent band can be seen at the dermo-epidermal junction which appears fragmented in the basal zone of the epiboly (↑) (× 270).

TABLE 1. Immunofluorescence findings using pemphigoid serum

Anatomical site	Result	Incubation times (h)				
		24	48	72	96	120
Explant	Positive	13	11	9	2	1
	Negative	0	0	0	0	0
Epiboly	Positive	0	2	1	1	1
	Negative	1	2	1	0	0

Intercellular fluorescence of the pemphigus type was found after incubation with pemphigus serum in all specimens save those that showed severe degenerative change (Table 2). The intercellular fluorescence was found both in the body of the cultured explant and in the migrating segments (Fig. 4).

Electron microscopy

Attention was particularly directed to the ultrastructural appearance of the dermo-epidermal junction at the site of the origin of the migrating epidermis. Beneath the body of the explant there was a normal junctional zone; the basal lamina was present and was normal in appearance. Normal anchoring fibrils were present in the immediately sub-epidermal connective tissue. The junctional zone beneath the main part of the migrating epidermis did not possess a basal lamina and there was no specialized arrangement of connective tissue bundles (Fig. 5). In two specimens (incubated for 48 h and 72 h respectively) there were fragments of a wispy, electron dense material beneath the migrating cells

TABLE 2. Immunofluorescence findings using pemphigus serum

Anatomical site	Result	Incubation times (h)				
		24	48	72	96	120
Explant	Positive	13	10	8	2	1
	Negative	0	1	1	0	0
Epiboly	Positive	10	8	8	2	1
	Negative	1	2	1	0	0

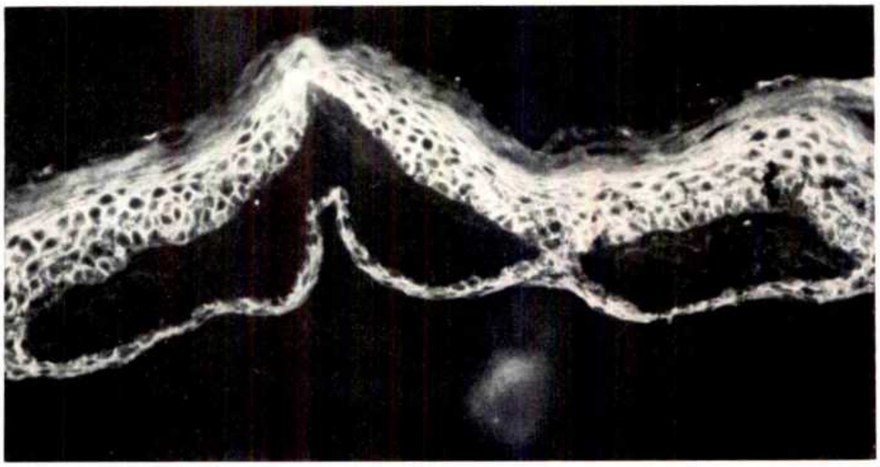

FIGURE 4. Immunofluorescence after 48 h of culture of skin explant and subsequent incubation with pemphigus serum; stained with FITC labelled anti-human IgG (× 135).

(Fig. 6). The basal lamina was present for a short distance 'beyond the shoulder' of the epiboly in the 72 h specimens (a distance of half to one basal keratinocyte). In two instances there was an expansion and fuzziness of the basal lamina at its termination at the point of origin of the migrating epidermal cells (Fig. 7).

The junctions between the spindle-shaped migrating keratinocytes consisted of interdigitating microvillous structures of the sort described by Marks, Bhogal & Dawber (1972).

DISCUSSION

The junctional zone is anatomically quite complex consisting of hemidesmosomes, basal lamina, anchoring fibrils and other specializations of connective tissue. Its components can be demonstrated quite easily but their demonstration sheds no light on their functional significance or site of origin. Our objective in combining a simple wound healing model with techniques demonstrating the basal zone has been to obtain information on the genesis of this region. In the course of maintaining

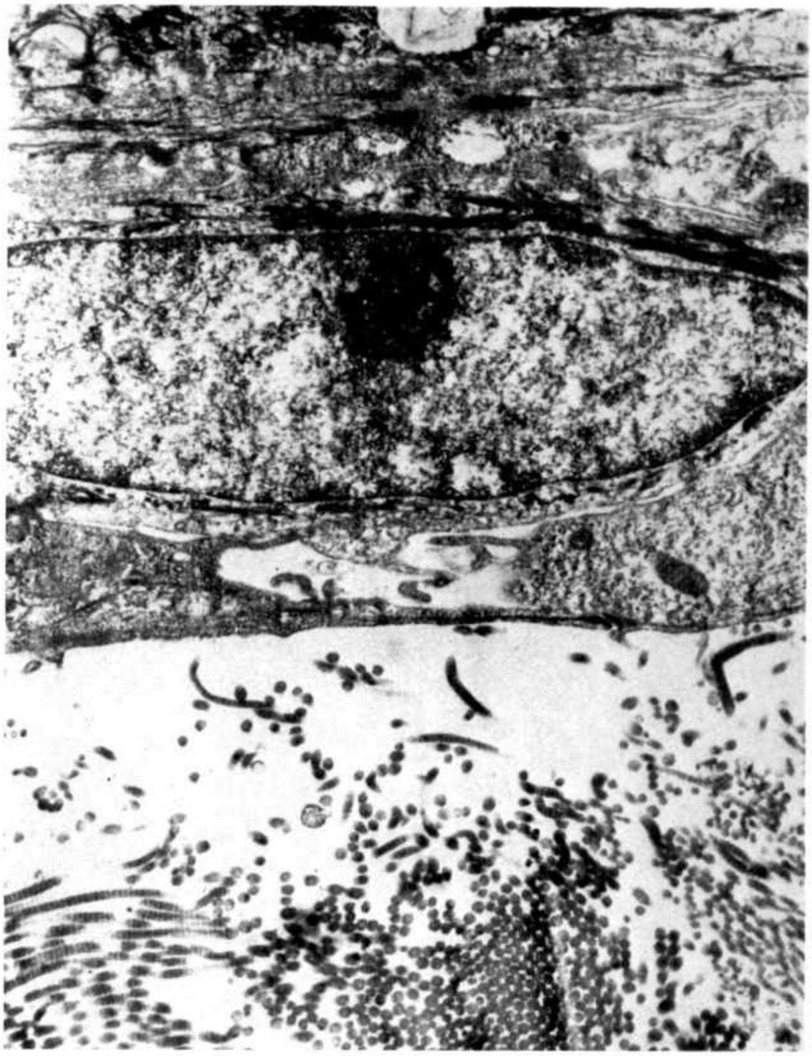

FIGURE 5. Electronmicrograph of area of migrating epidermis from 48 h culture of skin. There is no visible basal lamina and no specialized arrangement of connective tissue at dermo-epidermal junction (× 7500).

human skin *in vitro* there is a prominent early phase of migration. We were particularly interested to learn how an isolated sheet of epidermal cells that had migrated to cover a denuded area and which possessed no junctional apparatus developed a stable relationship with the underlying dermis in a relatively short period of time.

Other workers have confirmed that migrating epidermal cells do not possess a basal lamina (Krawczyk, 1971; Martinez, 1971). We were impressed with the way spindle shaped epidermal cells came into close contact with connective tissue fibres without any special sub-epidermal structure or organization. No component of the basal zone was demonstrable beneath migrating epidermal cells with any of the techniques, in specimens incubated for 24 h. Two specimens incubated for 48 h showed basal zone antigenicity in the 'epiboly'. Some skin fragments maintained for longer periods showed the same

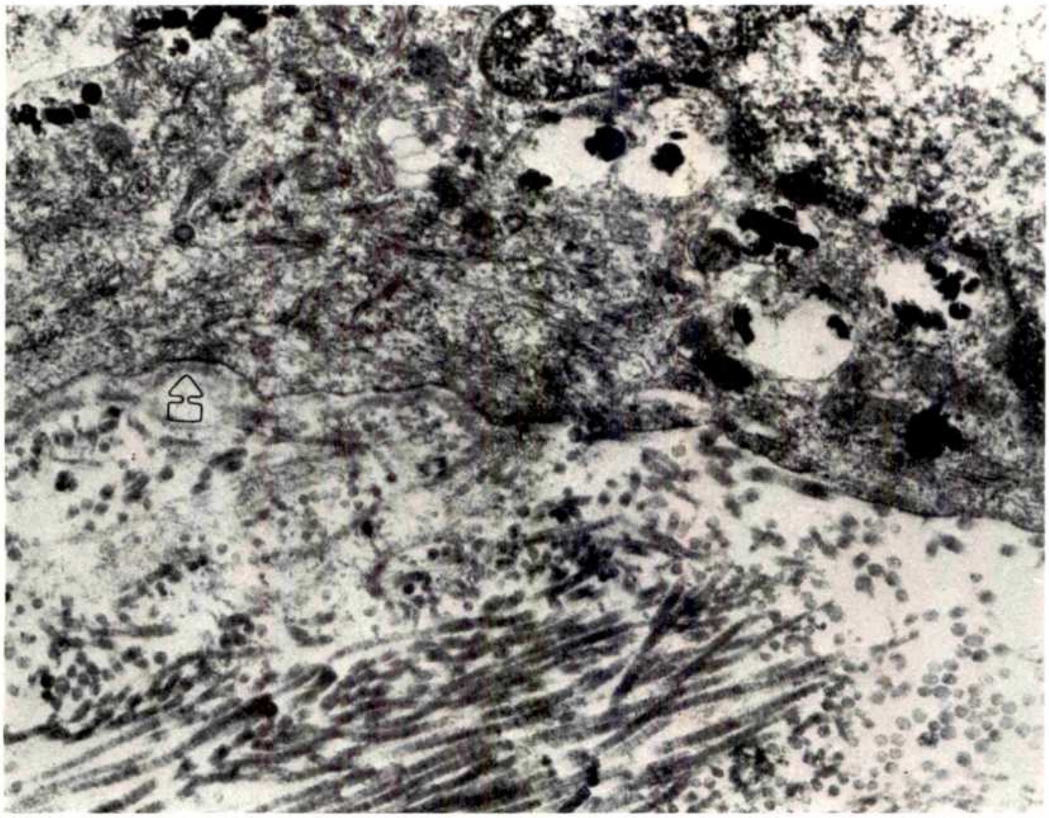

FIGURE 6. Electron micrograph of dermo-epidermal junction near junction of explant and migrating epidermis in 48 h culture of adult human skin. Fragments of wispy basal lamina can be seen beneath the epidermal cells (↑) (× 15,000).

distribution of antigenicity and it seems probable that if no degenerative changes were to occur then ultimately all parts of the sub-epidermal zone would contain the antigenic components of the basal zone. The ultrastructural observations also suggest that the basal zone is reconstituted after an initial 'lag period'. We were unable to account for the unusual appearance of the basal lamina at the apparent origin of the migrating epidermal cells. The wispy appearance of the basal lamina in the three explants in which it was observed beneath the migrating epithelium (two at 48 h and one at 72 h) suggests that it develops patchily rather than uniformly. The fact that it develops in close contact with migrating cells on a relatively acellular dermis suggests that it is produced by epidermal cells rather than by any dermal component. Sarkany & Gaylarde (1970) also observed the formation of new basal lamina beneath migrating epidermal cells from human skin explants. Basal lamina has been observed to develop from chick corneal epithelium on a substratum of dead chick lens (Hay & Dodson, 1973). Furthermore, ultrastructural studies of healing mouse epidermis suggested that epidermal cell re-orientation took place without an underlying basal lamina and that this structure developed from epidermis (Croft & Tarin, 1970).

It seems that the epidermis possesses a greater potentiality in the healing process than was once imagined. It is capable of a considerable degree of active movement independent of mitosis (Marks & Nishikawa, 1973); its individual keratinocytes can actively phagocytose debris, such as fibrin (Wolff &

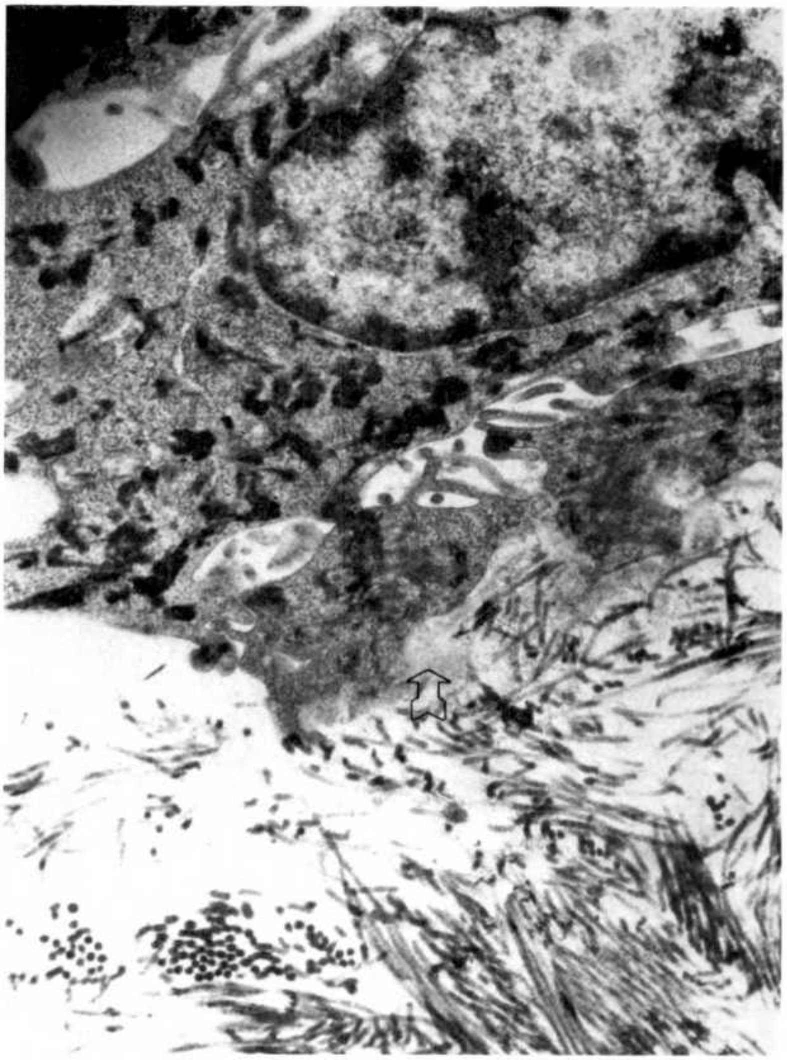

FIGURE 7. Electron micrograph of junctional area between explant and migrating epidermis in 24 h culture. An expansion and fuzziness of the basal lamina is visible at its termination at the point of origin of the migrating epidermal cells (↑) (× 10,000).

Konrad, 1972); and it can develop at least a considerable part (if not all) of its own dermo–epidermal junctional apparatus. Clearly re-epithelialization rather than connective tissue refashioning is of major importance in wound healing of skin.

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The removal and restitution of hair fat in the rat

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SUMMARY

Castrated male rats were washed with a solution of sodium lauryl sulphate and warm water using a standard technique. Examination of samples of hair clipped from one flank showed that about 50% of the total ether-extractable hair fat was removed. The rate of restitution of hair fat was then measured by clipping a further sample from the opposite flank of each rat after an interval. The plateau level was restored in about 8 days. There was no significant correlation with body weight either of the plateau level in unwashed rats or of the rate of build-up of lipid in washed rats. This result provides no justification for correcting levels of hair lipid for body weight.

Rats have a plateau level of ether extractable hair fat. In our colony this amounts to 17–18 mg/g of hair in immature or mature males, 16 mg in immature females and about 12 mg in mature females. The level may be reduced by washing with warm water and sodium lauryl sulphate, and the rate of restitution of lipid may be then studied by taking a sample of hair immediately after drying and another sample after an interval of 8 days. This procedure has been used as a measure of the activity of the sebaceous glands (Ebling, 1967, 1974; Ebling & Skinner, 1967). In the work to be described, the restitution of hair fat after washing was studied with the objects of determining the period for which the rate of increase was linear and of devising a shortened procedure for measuring sebaceous gland secretion.

MATERIALS AND METHODS

Animals

Male white rats from the random mated closed colony in the Sheffield University Department of Zoology were used. They were castrated at 4–5 weeks and the experiments were carried out at 13–16 weeks of age, when their body weights ranged from 170–284 g.

Experimental procedure

40 rats were shampooed by the following procedure. (a) Each rat was first acclimatized by immersing it up to the neck for 30 s in warm water (36°C). (b) The rat was then immersed for a further 10 s and 5 ml of 5% sodium lauryl sulphate solution was discharged (over 5 s) from a 20 ml plastipak disposable syringe without a needle. The shampoo was massaged well into the coat for 45 s. (c) The shampooing described in (b) was repeated three more times, making a total volume of shampoo used per rat of 20 ml. (d) The rat was rinsed for 1 min under a warm water tap.

After washing the rats were dried with a hair dryer. One to two grams of hair were immediately removed by clipping from the left flank. The hair was extracted successively with five 50 ml portions of diethyl ether; the extracts were combined in tarred aluminium foil cups and evaporated to dryness. After weighing, the extracted lipid was expressed in mg/g of hair.

The rats were put into clean individual metal boxes furnished with new sawdust. Ten rats had a further sample of hair clipped from the right flank after 1 day, ten after 2 days, ten after 4 days and ten after 8 days. Initial and final samples were also taken, and processed simultaneously, from one unwashed litter-mate of each experimental rat.

RESULTS

The mean plateau level in forty unwashed castrated rats was 7·73 mg lipid/g of hair, and the mean level in their newly washed litter mates was 3·99 mg/g; thus shampooing removed about half of the total ether extractable lipid (Table 1).

TABLE 1. Levels of hair fat in castrated rats (mg/g hair) immediately and at intervals after standard washing with 5% sodium lauryl sulphate, compared with levels in unwashed litter mates. Means ± s.e.

Unwashed control rats			Days after washing	Washed rats			
n	Initial level at day 0	Level when sampled		*n*	Initial level at day 0	Percentage of total lipid removed	Level when sampled
10	7·23 ± 0·22	7·23 ± 0·22	1	10	3·62 ± 0·13	50·0	4·20 ± 0·21
10	7·12 ± 0·27	7·21 ± 0·33	2	10	3·71 ± 0·17	47·9	4·98 ± 0·28
10	8·53 ± 0·31	8·62 ± 0·35	4	10	4·09 ± 0·28	52·1	5·98 ± 0·21
10	8·05 ± 0·33	8·37 ± 0·27	8	10	4·53 ± 0·17	43·8	8·39 ± 0·37
40	7·73 ± 0·17		Overall mean	40	3·99 ± 0·11	48·4	

There were significant, if relatively small, differences between some of the initial levels in the four groups, whether unwashed or washed. The differences between the unwashed groups appear meaningful, because there was no significant change in any group during the experimental periods of up to 8 days. The groups were not set up and processed contemporaneously, and the differences in initial plateau levels were probably due to differing environmental conditions prior to the experiment. It is unlikely that they were accounted for by possible differences in age, since there was no significant correlation between body weight and hair fat level (see below).

The mean levels in the washed rats immediately after shampooing appeared to be related to the plateau levels in their unwashed litter mates. An analysis of this relation gave a correlation coefficient of +0·271 (P = 0·1) and a regression coefficient of +0·178, which support this possibility even though they fail in statistical significance.

The rate of build-up of lipid depends on the balance between secretion and loss to the environment, and ultimately falls to zero at a plateau. While, after washing, the rate may be linear at first, it must eventually dwindle as the plateau is approached. The lower is the initial washed level, the longer will the build-up remain linear. Thus when rates are calculated from measurements made over a period

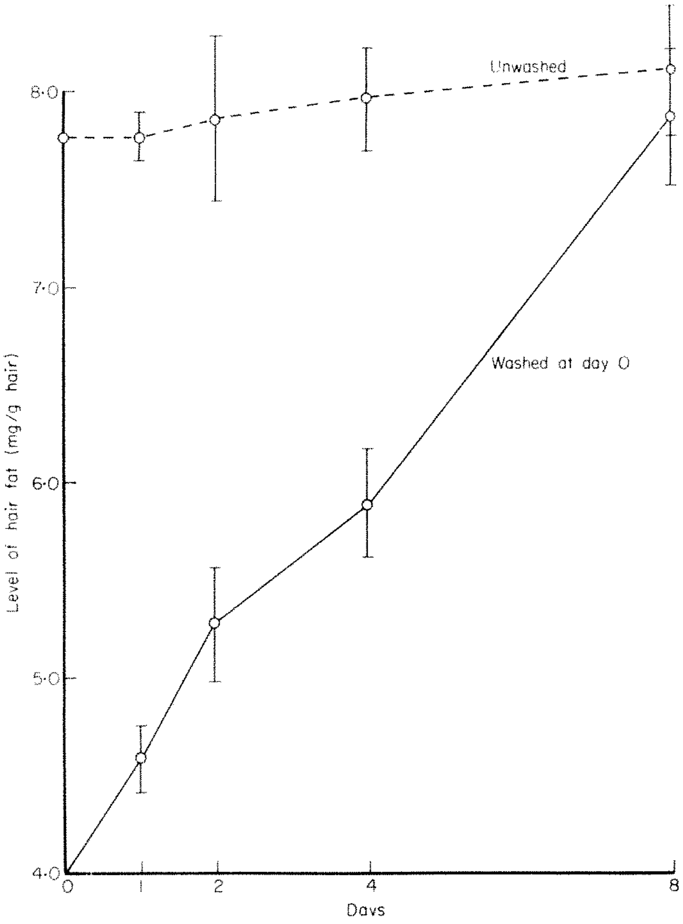

FIGURE 1. Changes in levels of hair fat (mg/g hair) in rats after washing in sodium lauryl sulphate (unbroken line) and in unwashed litter mates (broken line) over the same periods.

of several days, they could show some dependence on the initial washed level. An overall analysis of the relationship between the newly washed levels and the subsequent rate of increase in lipid, calculated per day for each rat, gave a correlation coefficient of −0·290 (P = 0·1–0·05) and a regression coefficient of −0·152. These figures suggest that the rate of build-up of lipid may show a slight inverse correlation with the initial washed level, but that the effect was small.

In the washed rats the hair fat approached the plateau after about 8 days (Table 1 and Fig. 1). The increases in hair fat over each period measured can be calculated for each rat by subtracting the initial level from the level when sampled (Table 2 and Fig. 1). No significant changes occurred in the unwashed control rats. In the washed rats the mean daily increases as measured over the 1 or 2 day periods were, respectively, 0·58 and 0·64 mg/g hair, and as measured over the 4 or 8 day periods 0·47 and 0·48 mg, that is about 20% less. These figures indicate that the rate diminishes as the plateau is approached.

The data can also be used to test whether the activity of sebaceous glands as determined by hair fat measurements is normally proportional to body weight. An analysis of the relationship between the plateau level and body weight for the unwashed rats gave a correlation coefficient of −0·184 and a

TABLE 2. Increases in hair fat (mg/g hair) after standard washing in sodium lauryl sulphate solution, compared with changes in unwashed litter mates. Means ± s.e. for ten rats in each group

Unwashed control rats	Days after washing	Washed rats
0·00 ± 0·12	1	0·58 ± 0·17
0·09 ± 0·42	2	1·27 ± 0·29
0·19 ± 0·26	4	1·89 ± 0·27
0·32 ± 0·33	8	3·86 ± 0·35

regression coefficient of −0.008. Between the daily increase and body weight in washed rats these values were −0·154 and −0·002 respectively. There was no significant positive relationship either between body weight and plateau level, or between body weight and rate of lipid accumulation after washing.

DISCUSSION

Our results indicate that the plateau level of ether extractable hair fat in castrated rats is less than half that of intact males. The mean figure of 7·73 mg lipid/g of hair was comparable with that obtained in previous experiments (Ebling, 1974). After washing with 5% sodium lauryl sulphate solution by a standard technique, the base level was reduced to a mean of about 4 mg lipid/g of hair. Subsequent restitution occurred at about 0·6 mg per day for the first two days and then at a lower rate, so that estimates of the daily rate based on measurements over 4 or 8 days were about 20% less than those based on periods of 1 or 2 days. These findings support the view of Shuster & Thody (1974) that 8 days is too long a period for calculation of a true daily rate of sebum production, but they do not, of course, invalidate any of the conclusions from experiments in which an 8 day period was used. The significant, even if relatively small, differences in plateau levels between groups of unwashed controls, emphasize the importance of standardizing environmental conditions and of using litter mates.

That neither plateau levels nor the rates of accumulation of hair fat after washing showed any positive correlation with body weight suggests that the correction of hair fat measurements for body weight is unjustifiable, notwithstanding the claim that total surface lipid as removed by immersion should be so adjusted (Shuster & Thody, 1974).

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The sebotrophic action of growth hormone (BGH) in the rat

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SUMMARY

Sebum production of rats was assessed by measuring the levels of fat extractable by diethyl ether from samples of hair clipped immediately after shampooing with sodium lauryl sulphate and 2 days later. By the use of matched litter mates it was unequivocally demonstrated that the response of the sebaceous glands to testosterone is virtually abolished by hypophysectomy and that it can be completely restored by pure bovine growth hormone.

Sebaceous glands are stimulated by testosterone, but for the full response in the rat the pituitary is necessary. When castrated rats were given testosterone, the sebaceous glands became larger (Ebling, 1948, 1957a), but the effect in hypophysectomized–castrated rats was insignificant (Lasher, Lorincz & Rothman, 1954, 1955; Ebling, 1957b). It has been claimed that a separate 'sebotropic' pituitary factor is responsible for permitting this response (Lorincz & Lancaster, 1957; Woodbury, Lorincz & Ortega, 1965a, b).

The necessity for the pituitary has been confirmed by experiments in which sebum production was measured, and restoration of the response to testosterone in hypophysectomized rats has been achieved with a growth hormone (Squibb) and a prolactin, though neither of these preparations had any independent effect (Ebling, Ebling & Skinner, 1969).

The concept of a 'permissive' pituitary hormone, that is to say a factor which enhances the response to testosterone without necessarily having any independent action has, however, been challenged. Whilst agreeing that treatment with testosterone results in a lower rate of sebaceous secretion in hypophysectomized–castrated rats than in castrated ones, Shuster & Thody (1974) propose that this is due to the subtractive effect of other hormones which act independently on the sebaceous glands. They point out that since thyroxine, and adrenal as well as testicular androgens, may all stimulate sebaceous secretion, a range of pituitary trophic hormones could indirectly but independently influence sebaceous secretion. In addition, they claim that a pituitary hormone, almost certainly an MSH, has a direct and independent effect on sebum production (Thody & Shuster, 1972, 1973). All these independent actions they describe as 'sebotrophic'. Fig. 1 illustrates the ways in which pituitary hormones can directly or indirectly influence sebaceous secretion. It attempts to alleviate

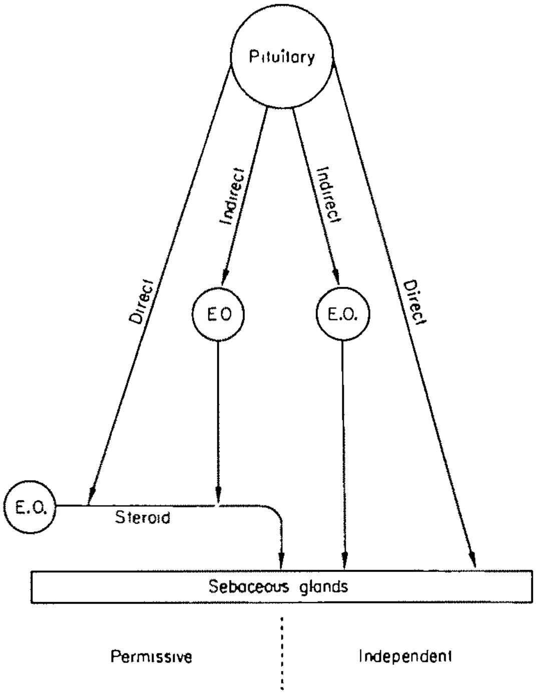

FIGURE 1. Possible ways in which pituitary hormones may affect the sebaceous glands. The effect may be direct or indirect, and in addition may be either independent or permissive, in the sense that the pituitary hormone facilitates the response to another hormone, for example a steroid. E.O., Endocrine organ.

any confusion which may have arisen by the application of the description 'sebotrophic', originally used only to describe 'permissive' or synergistic actions, also to independent actions.

Shuster & Thody deny the existence of any synergistic interaction between pituitary hormones and testosterone, maintaining that the respective effects are independent and additive. They believe that the incremental response due to testosterone is unaffected by removal of the pituitary (Thody & Shuster, 1970, 1971), and that any claim to the contrary results only from the use of methods which are inferior to their own (Shuster & Thody, 1974).

In the experiments to be described the effect on sebaceous secretion of a bovine growth hormone prepared by C.H.Li was investigated. Sebum production was measured in rats by a modification of the method originally proposed (Ebling & Skinner, 1967).

METHODS

Animals

Male albino rats from the randomly mated closed colony maintained in the Zoology Department of Sheffield University were used. All rats were castrated at 6–7 weeks of age. Rat cake (Oxoid Diet 86) and water were available *ad libitum* and all rats received a daily supplement of bread and milk. Animals were individually housed in metal boxes with sawdust from the start of treatment, and provided with clean quarters immediately after shampooing.

Design of experiments

Litter mate groups were used and each rat was given one of the following treatments: (i) untreated, (ii) testosterone, (iii) hypophysectomy and NaCl vehicle, (iv) hypophysectomy, testosterone and NaCl vehicle, (v) hypophysectomy and bovine growth hormone in NaCl vehicle, (vi) hypophysectomy, growth hormone and testosterone.

Hypophysectomy was carried out at 16–18 weeks, by the paratracheal route under ether anaesthesia. Completeness was judged by: (a) continuing loss of body weight, (b) low adrenal weight post mortem, and (c) macroscopic examination of the sella.

Testosterone was implanted subcutaneously, in the form of cylinders weighing approximately 10 mg, about 2 weeks after hypophysectomy. When the rats were killed 10 days later the implants were removed and re-weighed. The loss of weight is shown in Table 1. The uptake per rat was between 0·2 an do·3 mg/24 h, equivalent to about 0·1 mg/100 g body weight/24 h. There were no significant differences in uptake between the groups.

TABLE 1. Total uptake (mg) over 10 days from testosterone implants. Means ± s.e.

	Castrated	Hypophysectomized –castrated	Hypophysectomized–castrated +growth hormone
per rat	2·65 ± 0·10	2·18 ± 0·06	2·50 ± 0·21
per 100 g body wt.	0·94 ± 0·04	1·03 ± 0·05	1·11 ± 0·10

Bovine growth hormone (BGH) was supplied by Professor C.H. Li (Hormone Research Laboratory, University of California, San Francisco). 0·5 mg in 0·25 ml 0·9% NaCl solution in 0·01 M NaOH was injected subcutaneously once daily for 10 days, starting about 2 weeks after hypophysectomy.

Measurement of sebum production

Sebum production was assessed by measuring the increase of ether-extractable hair fat over a 2 day period, following washing with sodium lauryl sulphate 8 days after the start of treatment. The technique (Ebling & Skinner, 1975) was modified from Ebling & Skinner (1967).

TABLE 2. Levels of ether extractable hair fat in samples of hair taken immediately after washing with sodium lauryl sulphate solution and 2 days later. Means ± s.e. for groups of eleven rats

	Castrated		Hypophysectomized–castrated			
	Untreated	Testosterone	Untreated	Testosterone	Growth Hormone	Growth hormone + testosterone
Immediately after washing	3·81 ±0·17	5·11 ±0·16	4·66 ±0·22	4·95 ±0·15	4·25 ±0·18	5·08 ±0·19
2 days later	4·74 ±0·26	6·86 ±0·23	5·24 ±0·18	5·63 ±0·26	5·27 ±0.27	7·40 ±0·24

RESULTS

The mean levels of ether extractable hair fat immediately after washing and 2 days later are shown in Table 2. The washed level was significantly higher in the testosterone treated castrated rats than in the unreated animals and the highest level in the hypophysectomized–castrated rats occurred in the group treated with both growth hormone and testosterone. These groups also obviously showed the greatest increases over the 2 day period. It must be pointed out that the higher initial levels in these groups will have reduced rather than exaggerated the differences between them and other groups with a slower build-up of hair fat.

The actual increases in hair fat over the 2 day period are shown for each rat in Table 3. For purposes of comparison with previously published results, the mean figures are more suitably expressed as mg lipid/g hair/24 h as in Fig. 2 and Table 4.

TABLE 3. Actual increases in hair fat (mg/g hair) in eleven litters of six rats each over the 2 day period immediaetly after washing with sodium lauryl sulphate solution

	Castrated		Hypophysectomized–castrated			
Litter no.	Untreated	Testosterone	Untreated	Testosterone	Growth hormone (BGH)	Growth hormone (BGH) +testosterone
1	0·7	1·7	0·5	1·2	1·7	1·7
2	1·4	1·7	0·4	1·0	1·4	2·2
3	1·0	1·6	1·1	1·3	0·8	2·4
4	1·4	2·0	0·6	0·4	0·8	1·9
5	1·3	1·6	1·0	0	1·0	2·5
6	0·7	1·1	0	0·2	1·5	2·8
7	1·8	2·4	0·5	0·9	1·0	2·9
8	1·0	2·4	0·5	−0·4	1·5	2·4
9	0·9	2·5	1·0	1·5	1·3	2·6
10	0·1	1·1	0·9	0·4	−0·3	1·2
11	−0·1	1·2	−0·2	−0·1	0·6	1·8
Mean	0·93	1·75	0·57	0·58	1·03	2·22
±s.e.	±0·171	±0·156	±0·125	±0·191	±0·169	±0·156
Compared with		Castrated	Castrated	Hypophysectomized –castrated	Hypophysectomized –castrated	Hypophysectomized –castrated + BGH
t		3·58	1·68	0·04	2·16	5·17
P		$<0·002$	NS	NS	$<0·5$	$<0·001$

It is possible to take out any variation between litters by subtracting from the figure for each treated rat that for the appropriate litter mate control. The mean increment due to each treatment can then be calculated. To determine whether this is significantly different from zero, that is whether the treatment had any effect, Student's t can be derived by dividing the mean by its own standard error (Table 5). A further advantage of this procedure is that it is possible to make direct comparisons between pairs, so that the relative effects of testosterone in castrated rats, hypophysectomized–

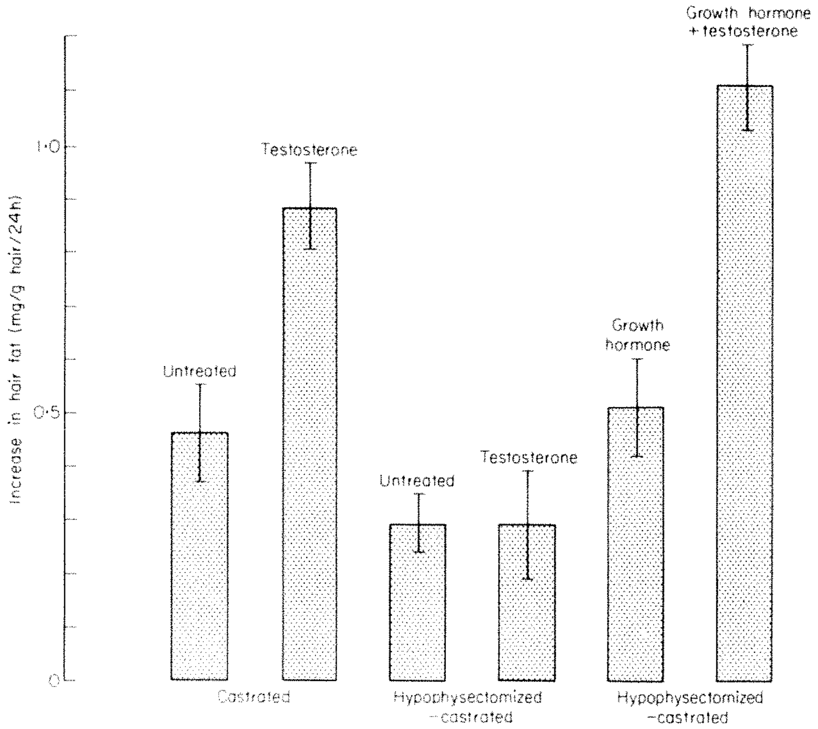

FIGURE 2. Sebum production (mg/g hair/24h) as measured by increase in hair fat (mg/g hair/24 h). Means ± s.e.m for groups of 11 rats.

TABLE 4. Sebum production as measured by increase in hair fat (mg/g hair/24 h) with and without individual correction for body weight. Means ± s.e. for groups of 11 rats

	Castrated		Hypophysectomized–castrated			
	Untreated	Testosterone	Untreated	Testosterone	Growth hormone	Growth hormone + testosterone
Sebum production mg/g hair/24 h	0·46 ± 0·09	0·88 ± 0·08	0·29 ± 0·06	0·29 ± 0·10	0·51 ± 0·09	1·11 ± 0·08
Sebum production mg/g hair/24 h/ 100 g body wt.	0·17 ± 0·03	0·31 ± 0·03	0·14 ± 0·03	0·14 ± 0·04	0·21 ± 0·04	0·49 ± 0·04

castrated rats, and hypophysectomized–castrated rats treated with growth hormone (BGH) can be assessed.

Sebum production in hypophysectomized–castrated rats appeared lower than in castrated rats (Fig. 2.) though the difference just failed to prove statistically significant at the 0·05 probability level (Table 3), even after removal of litter variation (Table 5). Treatment of hypophysectomized–castrated rats with bovine growth hormone restored the level to that of castrated rats. The increase was just significant (Table 3), though slightly less so when litter variation was removed (Table 5).

TABLE 5. Effects of various treatments on changes in hair fat. The increments, in mg lipid/g hair/24 h, were calculated by subtracting the figure for the appropriate control rat in each litter from that for the treated rat. Means and s.e. are each for 11 pairs of rats

	Effect of hypophysectomy	Effect of growth hormone in hypophysectomized–castrated rats	Effect of testosterone		
			In castrated rats	In hypophysectomized–castrated rats	In hypophysectomized–castrated rats +BGH
Increment	−0·18±0·09	+0·22±0·12	+0·40±0·07	−0·03±0·09	+0·60±0·08
t	1·99	1·86	6·08	0·36	7·82
P	0·1–0·05	0·1–0·5	<0·001	n.s.	<0·001

Testosterone very significantly increased sebum production in castrated rats to nearly twice that of untreated controls (Fig. 2, Tables 3 and 5). In contrast, it failed to produce any detectable effect in hypophysectomized–castrated rats. A direct comparison between the mean increments produced in castrated and in hypophysectomized–castrated rats (see Table 5) gives $t = 3{\cdot}96$, $P < 0{\cdot}01$. The response was completely restored by bovine growth hormone; a comparison of the increments produced by testosterone in hypophysectomized–castrated rats treated with growth hormone with matched untreated controls gives $t = 5{\cdot}39$, $P < 0{\cdot}001$. Indeed, the response was marginally greater than that in castrated rats with intact pituitaries. Finally, it may be noted that none of these conclusions about the relative responses to testosterone is materially affected by adjustment of the figures for body weight, as proposed by Shuster & Thody (1974).

DISCUSSION

Our results demonstrate unequivocally not only that hypophysectomy virtually abolishes the response of the rat sebaceous glands to testosterone, but also that a pure bovine growth hormone can restore it.

The evidence emphatically contradicts the view of Thody & Shuster (1971) that 'at physiological doses the action of testosterone propionate is independent of the pituitary'. Their published evidence needs, therefore, to be examined in some detail. They used only five rats in each group and they were not litter matched. Two groups of animals were untreated, and the others received testosterone propionate given subcutaneously in isopropyl myristate, with the following results:

Dose (mg/day)	Surface fat (mg/4 days)	
	Castrated	Hypophysectomized–castrated
0	99·2 ± 4·8	36·1 ± 4·3
0·05	93·5 ± 3·1	52·1 ± 1·9
0·5	149·9 ± 16·1	72·0 ± 7·9
5·0	181·3 ± 25·3	90·0 ± 10·7

The authors do not dispute that the levels are throughout lower in the hypophysectomized–castrated rats than in the corresponding castrated animals. Their argument is that the mean increment due to testosterone propionate is the same whether or not the rats are hypophysectomized. The increments produced can be calculated as:

Dose (mg/day)	Castrated	Hypophysectomized–castrated
0·05	− 5·7	+ 1·60
0·5	+ 50·7	+ 35·9
5·0	+ 82·1	+ 53·9

With the exception of the lowest dose, the increments in the hypophysectomized–castrated rats are, in fact, not equal to those of the castrated rats, but less. The authors' statement that the action of testosterone is independent of the pituitary is thus based solely on the figures obtained for the smallest of the doses (0·05 mg) which appeared to produce a significant effect in the hypophysectomized–castrated rats but not in the castrated ones. This finding must be viewed with suspicion for two reasons. First, the dose of 0·05 mg/day is almost certainly less than physiological and on the authors' own admission had no effect on preputial gland weight. Secondly, the effect may have been due to the injected vehicle (isopropyl myristate) rather than the testosterone, since the controls received no injection of any kind. We have, indeed, noted just such an increase after subcutaneous injection of arachis oil to hypophysectomized–castrated rats (Ebling, 1974). To avoid any misunderstanding of the issue at stake, we do not deny that the sebaceous glands may show a small response to testosterone after hypophysectomy, as Nikkari & Valavaara (1969, 1970a, b) have found. When the results from a number of experiments were grouped together, it was possible by matching each of 44 hypophysectomized–custrated rats treated with testosterone against an untreated litter mate to show a significant effect, about 20% of that produced in rats with intact pituitaries (Ebling, 1974). However, we believe that the permissive or synergistic role of a pituitary hormone in mediating the response to testosterone is established by our evidence, and that Thody and Shuster's often repeated challenge is based on a misinterpretation of their own data. Finally, the demonstration that a pure growth hormone can completely restore the response of the sebaceous gland to testosterone, as well as possibly exert an independent effect, does not lend support to the view (Thody & Shuster, 1973) that the MSH's are the only so-called 'sebotrophic' factors of the pituitary.

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Mites in the environment of cases of Norwegian scabies

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SUMMARY

Three cases of Norwegian scabies diagnosed in Glasgow hospitals within 1 year each caused widespread outbreaks of papular eruptions amongst the patients and staff, followed later by a few cases of classical scabies. Symptoms and timing suggested that these infections were caused mainly by immature mites derived by personal contact and possibly by contact with infected cuticular fragments which were abundant in the environment of the three patients. To control a ward epidemic, isolation of the Norwegian scabietic should be followed by environmental and then by personal disinfestation.

Norwegian or crusted scabies has usually been regarded as rare (Calnan, 1950; Wells, 1952) but the increased incidence of cases reported during recent years (Martin-Scott, 1957; Maguire & Kligman, 1960; Herridge, 1963; Haydon & Caplan, 1971; Wishart, 1972; Paterson, Allen & Beveridge, 1973; Evans, 1973; Ferguson, 1973) suggests that it may be more common than has been supposed. Diagnosis, if suspected, is not difficult, but cases may remain undetected for long periods, particularly when admitted to general wards.

Three cases have been found in wards in hospitals in the Glasgow area during the last year and each showed the characteristic high infectivity of this disease. Skin eruptions developed extensively amongst nursing and medical staffs and amongst other patients in the wards and several instances of typical scabies resulted.

The significance of infestation of the personal environment by mites in the development and aggravation of skin eruptions has recently been attracting interest (Hewitt, Walton & Waterhouse, 1971; Hewitt *et al.*, 1973) so a special examination of the surroundings of each of the present patients was made.

MATERIALS AND METHODS

Case (1) December 1972: female, aged 66 years.

Several patients in a dermatological ward complained of irritating papules about the ankles, on the inner aspect of the upper thighs and on the buttocks on 30 December; one case of scabies was detected with burrows present and a live specimen of *Sarcoptes scabiei* was isolated on 19 January 1973. Neither burrows nor mites were found on other patients. Members of nursing and medical staffs next complained of irritating papules on the anterior aspect of the forearms and in some cases about the ankles and on the inner aspect of the thighs. In one case a living mite was removed from a papule on the anterior aspect of the wrist but no burrows were seen. An infant that had been in the ward for several weeks was then found to have a burrow on the sole of the foot and from this a mite was obtained. As further cases affected by scattered irritating papules kept developing the ward was evacuated on 11 March.

In retrospect, it was considered that there must have been a case of Norwegian scabies in the ward and suspicion fell on a woman patient aged 66 years, who was suffering from psoriasis and carcinoma of the bronchus. This patient had been admitted to the ward on 20 December 1972 and the first complaint of irritating papules by other patients had occurred 10 days later. Her bed was next to that of the patient on whom the mite was found on 19 January and she had been examined for scabies but no scrapings had been taken. She was the only patient never to complain of irritation but unfortunately died of carcinoma the day after the ward was evacuated and before her probable infestation had been diagnosed.

Dust samples were taken from various points in the ward and adjoining rooms by means of a suction device designed for the study of populations in house dust (Sesay & Dobson, 1972). These samples were cleared in 90% aqueous lactic acid containing lignin pink stain and the mites were identified and counted. The first observations were exploratory and not strictly quantitative but they revealed the presence of heavy infestations of *S. scabiei* on used bed linen and on soft furnishings in the adjacent day and sister's room. Curtains, especially those round the suspect patient's bed and those in the day room on which only immature stages were found, floors, walls and children's toys all showed infestation.

Samples were then taken from two bags containing used bed linen and the numbers of *S. scabiei* (excluding eggs) per g of dust were estimated. One sample from the bag containing the suspect patient's linen was conspicuous for the large amount of epithelial and crust material it contained. It showed a population density of 6312 mites/g. The sample from the other bag contained little skin debris but still showed an infestation of 472 mites/g. All developmental stages, from eggs to ovigerous females, were abundant in these samples and in the first one a considerable proportion of the mites were embedded in crust particles and scales. Some of the infested material was incubated on agar plates at 30°C for several days but no living mites were seen. The samples were, however, not taken until 2 weeks after the ward had been closed and the infestation had probably died out.

The medical and nursing staff of the affected ward were also responsible for a male ward two floors below. No male patients became affected, however, and dust samples from soiled linen and from an upholstered chair in the day room of this ward showed no *S. scabiei*.

Treatment of patients and staff with benzyl benzoate emulsion was ineffective prior to evacuation of the infested ward. Patients, nursing and medical staff returned after 21 days but no further cases developed.

Case (2) April 1973; female, aged 91 years.

In April 1973 nursing staff in a psychiatric ward in another hospital complained of irritating papules on the forearms. Occasional cases of scabies had previously occurred in this ward but the staff had not been affected. All patients were examined; several complained of irritating papules on legs, thighs and buttocks but no burrows were found; two showed typical scabies with well marked palmar burrows. One patient with senile dementia admitted on 24 February 1971, while not complaining of irritation, showed hyperkeratosis about the palmar creases and around the bases of the nails. Scrapings from this patient showed many eggs, empty shells and immature mites. Only minute dust samples could be obtained from the bedding in this instance but an infestation density of about 200 *S. scabiei*/g was recorded.

The patient with Norwegian scabies was isolated but died about 3 days later. The two with scabies were given routine antiscabietic therapy and the nursing staff were advised to apply calamine lotion. No further cases of scabies developed and no more members of the nursing staff became affected.

Case (3) October 1973; male, aged 73 years.
This case was admitted to a general medical ward on 20 October 1973 following a minor cerebrovascular incident. The patient also suffered from a generalized pruritic condition. On 30 October, Norwegian scabies was suspected and scrapings showed a multitude of eggs and a few mites. Scales from this patient examined within 24 h showed mainly eggs and very few mites but on re-examination after a week at room remperature many mites and relatively few unhatched eggs were present. The mites, however, appeared to be dead.

At this time, i.e. 30 October, five members of the nursing staff and two patients complained of irritating papules on legs, thighs and forearms, but no burrows were seen and no mites were found. Two weeks later two patients from neighbouring beds developed typical scabies and mites were recovered from one of them. The patient with Norwegian scabies was isolated and later transferred.

Dust sampling indicated heavy infestation by *S. scabiei* of the patient's sheets (900–2154/g), of the floor and floor cracks around his bed (840/g), of his screening curtains (666/g) and of two nearby chairs (333/g).

These densities, though measuring the degree of infestation of the dust, give no idea of the total numbers of mites being disseminated by a patient with Norwegian scabies. To get some idea of this the patient's soiled sheets, pillow slips, pyjamas and nightshirts were accumulated for about 2 days, fumigated with ethyl acetate and then washed in warm (40°C) soapy water in a 'Bendix' washing machine for ten minutes. All the effluent from this wash, from a warm rinse in the same machine and from a spin dryer was passed through a sieve of aperture 105 μm and the sediment collected. This contained masses of skin debris, all stages of mites and fibrous matter from the fabrics. Small portions of the sediment were then weighed and, after being cleared in lactic acid and lignin pink, were assessed for mites. It was estimated that the total sediment contained about 7640 mites (excluding numerous eggs) this being equivalent to a density of 2570/g dry wt.

DISCUSSION

The cases reported here resemble those recorded previously in that hospitalized patients with Norwegian scabies gave rise to numerous cases of atypical scabies amongst other patients and hospital staff (Wells, 1952; Herridge, 1963; Wishart, 1972). At first, complaints were of irritating papular eruptions on forearms, thighs and other parts not usually associated with burrowing scabies mites, but several weeks later typical symptoms developed with ovigerous females burrowing at classical sites.

According to Mellanby (1972) infection by *S. scabiei* is usually accomplished by a newly fertilized female which forms a burrow at one of the sites of predilection. She never normally leaves this burrow and may lay eggs in it at the rate of 2–3/day for nearly 2 months. The young mites leave the parental burrows, move across the skin and enter hair follicles, and the follicular papules found near the burrows and on other parts of the body are probably a result of their activity. Mortality amongst immature mites is high and experimental attempts to produce infection with eggs and immature mites succeeded in producing only crops of papules. It seemed probable therefore that under normal circumstances too few of these stages would survive to produce a permanent infection. If, however, large numbers of immatures were picked up, then there would be a greater chance that some burrows would be established.

Our observations show that the environment surrounding a case of Norwegian scabies becomes contaminated with mites of all stages. Many of these occur freely amongst the abundant epithelial debris but others are enclosed in the remains of burrows within this material. Free mites do not survive long off the host (Munro, 1919; Mellanby *et al.*, 1942) and it seems likely that most of those found will be dead. In the warm bedding and on the clothes and body of the patient, however, there may be many active free mites and these may transfer to the arms and other exposed surfaces of nurses and medical staff handling him. Because of the high mortality during development, the surface activities of the young mites and the reluctance of established females to leave their burrows, most of the mites transferred will be immature and potentially capable of causing follicular papules on the affected parts. The eggs and mites remaining in the cast-off cuticular fragments will, unlike the free mites, be somewhat protected from desiccation and may survive and remain infective for some time. Eggs in scales scraped from patient (3) hatched when kept at room temperature and Gerlach (1857) showed that eggs removed from skin and protected from high temperature and desiccation could remain alive for at least 10 days. Persons coming into contact with these widely scattered skin fragments may thus also acquire an infection of immature mites and develop follicular papules.

We suggest, therefore, that the atypical cases of scabies which occur in ward epidemics associated with Norwegian scabies are at first cases of localized follicular irritation caused by immatures of *S. scabiei*, and that persons thus affected avoid the first stage of a normal infection i.e. the burrowing of the fertilised female mite. The subsequent appearance of typical scabies would then depend on enough of the mites surviving to enable fertilized females to be produced so that, in most instances, where the patients were active the infection would tend to die out. In bed-ridden patients, however, where the relatively warm and undisturbed skin environment favours survival of the mites, typical scabies might develop after 2–3 weeks.

The concentration of infective material is greatest around the bed of the patient with Norwegian scabies and patients in adjacent beds are most likely to be infected. In case (1) the patient in the next bed developed papules within 10 days of the admission of the Norwegian scabietic and typical burrows appeared 3 weeks later. In case (3), two patients in the beds next to that of the Norwegian scabietic developed burrows about 3 weeks after his admission.

Mites and infective particles are probably dispersed in several ways. Medical and nursing staffs become infected and, as suggested by Wells (1952), may help to distribute material within a ward. No direct examples of this were noted in the present observations, but Paterson *et al.* (1973) noted the correspondence of papules on the forearms of nurses with those on the chest and axillary folds of patients requiring frequent lifting. In case (1) the medical and nursing staff moved freely between the infested female ward and the male ward yet the latter remained uninfested. Possibly routine hygiene sufficed to prevent transfer of infestation by this means. Fomites such as bed-clothes and towels are obviously suspect and there may be some measure of aerial dispersion. Cunnington & Gregory (1968) demonstrated that house dust mites become air-borne during bed making operations and this probably also applies to the similar sized scabies mites. The infestations observed on curtains in cases (1) and (3) are evidence of this mode of dissemination.

To control a ward epidemic it is necessary to identify and isolate the case of Norwegian scabies. Bedding, curtains and soft fabrics must then be laundered and floors, walls and hard surfaces sprayed with an acaricide, e.g. 1% B.H.C. (Lindane) emulsion. Where this can be done, the ward may be re-occupied within a few hours. Should this be impracticable, disinfestation can be achieved by thorough cleaning and closure of the ward for 2 weeks. Treatment of the affected patients and staff with anti-scabietic measures will be ineffective, and probably should be avoided, until they can be removed from the infested environment. Repeated anti-scabietic treatment, without removal from an infested environment, is likely to lead to a dermatitis venenata. Once removed from the environment, it is advisable to give all cases, including the atypical, anti-scabietic treatment.

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Pharmacology and Treatment

High dose ascorbic acid in the management of thalassaemia leg ulcers—a pilot study

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SUMMARY

Eight patients with β thalassaemia major suffering from leg ulcers, were treated over an 8-week period with 3 g ascorbic acid daily in a controlled double-blind crossover study. The ulcers of all the patients showed a high rate of either complete or partial healing.

A recognized feature of hereditary haemolytic anaemias is the increased incidence of chronic leg ulcers, although these are unusual in severe cases of β thalassaemia major that die in childhood. Leg ulcers are, however, a therapeutic problem if they develop in this disease in patients who have an unusually mild clinical and haematological picture compatible with survival into adult life. Although bed rest and antiseptic dressings to the ulcer may encourage healing, the present treatment of leg ulcers in such patients with β thalassaemia major is unsatisfactory.

It has been recognized for many years that ascorbic acid deficiency is associated with delayed wound healing (Bartlett, Jones & Ryan, 1942). Infections and other forms of stress accelerate vitamin C depletion and, even in the absence of clinical evidence of scurvy, ascorbic acid has been recommended for the treatment of indolent ulcers (Tobias, 1963).

This paper reports the results of giving 3 g of ascorbic acid daily for 8 weeks to eight patients who suffer from β thalassaemia major complicated by multiple leg ulcers. The study was run in a controlled double-blind crossover fashion and the results reported in terms of either complete healing or, alternatively, partial healing if there was a reduction in the size of the ulcer to less than half of its original diameter.

MATERIALS AND METHODS

The eight patients (five male and three female) in this trial were siblings of two large related families. The average age of the patients was 25 years, the youngest being 18 and the oldest 33 years. In both families the two parents had been proven to have β thalassaemia minor with elevated haemoglobin A_2 levels. The haemoglobin levels of the eight patients ranged from 7·8 to 8·8 g/100 ml with fetal haemoglobin levels between 12% and 24%. All the patients had hepatosplenomegaly and had two to four leg ulcers each for at least 2 years prior to the trial. These ulcers, ranging in size from 2–4 cm

Requests for reprints to Dr Huntsman.

diameter, were in the area of the internal and external malleoli, the skin surrounding them being indurated and pigmented.

The eight patients, who were all of high social class and receiving good mixed diets, were randomly divided into two groups. During the first 8 weeks all patients in group 1 received 3 g of effervescent ascorbic acid (Roche) daily in divided doses whilst all patients in group 2 received 3 g of a placebo effervescent tablet of identical appearance. During the second eight-week period, this regime was reversed, group 2 receiving the ascorbic acid (3 g daily) whilst group 1 received the placebo tablets. The dimensions of the ulcers were measured during weekly visits to an out-patient clinic, neither the patient nor the medical attendant knowing the true nature of the tablets until the completion of the 16 week trial.

During this trial period the patients were instructed to raise their legs into a horizontal position for 14 h, including sleep, per day. This regime was found to be compatible with the patients' normal working and domestic life.

RESULTS

The ascorbic acid was well tolerated by all patients. One patient reported mild 'heartburn' and two others noted the diuresis associated with high doses of ascorbic acid.

TABLE 1. Group 1

		Ascorbic acid medication (8 weeks)		Placebo following ascorbic acid medication (8 weeks)		
Patients	Number of leg ulcers	Totally healed	Partially healed	Totally healed	Partially healed	Unhealed ulcers at end of trial
1	4	2	1	3	1	1
2	3	2	1	3	0	0
3	3	1	1	2	1	1
4	4	2	2	3	1	1
Total	14	7	5	11	3	3

Tables 1 and 2 demonstrate that in the first 8 week period of the study, seven of the fourteen leg ulcers in the four patients in group 1 were completely healed and 5 partially healed whilst receiving 3 g ascorbic acid daily. This contrasts with the fact that of the twelve leg ulcers in the four patients receiving placebo tablets in group 2, only four were partially healed in the same 8 week period. (This difference is highly significant, $P < 0{\cdot}01$.) However, during the succeeding 8 week period when the four patients in group 2 were each receiving 3 g ascorbic acid daily, nine of their twelve ulcers were totally healed. It will be noted from Table 1 that a further four ulcers possessed by the four patients of group 1 healed completely during the second 8 weeks period when they were receiving placebo tablets only.

DISCUSSION

The patients studied in this trial were suffering from β thalassaemia major which had sufficiently mild clinical manifestations to permit them to carry out virtually all normal life activities. Indeed, their major disability was not due to their anaemia but to the frequent attacks of pain resulting from recurrent infection of their ulcers.

TABLE 2. Group 2

Patient	Number of leg ulcers	Placebo prior to ascorbic acid medication (8 weeks)		Ascorbic acid medication (8 weeks)		Unhealed ulcers at end of trial
		Totally healed	Partially healed	Totally healed	Partially healed	
1	3	0	1	2	1	1
2	2	0	1	2	0	0
3	3	0	1	2	1	1
4	4	0	1	3	1	1
Total	12	0	4	9	3	3

During the 16 week period of this study all patients were told to continue their normal antiseptic dressings procedure for local ulcer care, and to raise their legs daily for 14 h. The effects of these additional therapeutic measures had been previously demonstrated to be relatively inefficacious when used on their own to promote leg ulcer healing. For example, two of the patients had been previously admitted for in-patient hospital treatment for their leg ulcers which included bed rest and local antiseptic dressings but no ascorbic acid medication. During 20 weeks each patient had healed two of their four ulcers, one other ulcer healing in one patient after a further 4 weeks hospital in-patient treatment.

Ascorbic acid is essential for the proper formation of collagen by fibroblasts, and there is evidence that it participates in the production of inter-cellular material in other tissues including the walls of blood vessels (Pauling, 1970). Whilst there was no reason to suspect a subscorbutic state in these patients, who were all of high social class, further work should include individual vitamin C studies. Lewin (1973) has stressed the importance of the ability of ascorbic acid to lower interfacial tensions and this conceivably would affect capillary blood flow. High doses of ascorbic acid would be therapeutically effective in these circumstances even if the patient had a normally acceptable vitamin C intake. Ascorbic acid medication is both cheap and non-toxic. The favourable results obtained in this pilot study suggests that it may have a role to play in the management of chronic leg ulcers in β thalassaemia major.

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Oral retinoic acid as therapy for congenital ichthyosiform erythroderma

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SUMMARY

Oral retinoic acid has been therapeutically beneficial for three patients with congenital ichthyosiform erythroderma and one with congenital bullous ichthyosiform erythroderma. Response to therapy was slow but definite with suppression of scale formation, marked reduction of painful cracking of the underlying epidermis and disappearance of skin irritation. Side effects have not occurred. Therapy had to be maintained to prevent recurrence of symptoms.

The management of congenital ichthyotic skin diseases has always been a frustrating therapeutic problem for dermatologists. In the past topical salicylic acid preparations (Baden & Alper, 1973) have been the mainstay of therapy because of their keratolytic activity, but such treatment was not directed at the basic problem—persistent scale formation. Large doses of retinol orally have been found partially helpful, but serious side effects frequently led to premature termination of this medication. More recently topical retinoic acid (Mirrer & McGuire, 1970; Gunter, 1972; Schorr & Papa, 1973) has shown promising results in various dyskeratotic skin diseases. However, inconvenience of application and frequent skin irritation, even if mild, have limited its acceptability for many patients. Hence we have tried oral retinoic acid. Its advantages are (1) easy administration; (2) no cutaneous irritation; (3) minimal side effects, and (4) satisfactory improvement.

CASE REPORTS

Case 1

R.F., a 27-year-old Frenchman. Since birth, he has had a generalized scaling skin eruption with marked ridging of the skin in the body folds. A diagnosis of congenital ichthyosiform erythroderma was made and he was commenced on retinoic acid, 10 mg capsules four times a day in November 1972. Within 3 weeks he had complete suppression of scale formation and had a residual slightly dry skin and mild ectropion. He had no complaints of headache, visual disturbance (with fundoscopic control), or cheilitis. Frequent liver, bone marrow and kidney studies were always normal. Within 3 weeks of stopping the medication during the summer of 1973 there was a reappearance of the characteristic scales and ridging of accumulated keratin on his limbs and abdomen. The patient is a healthy young man, who found it too troublesome to take capsules four times a day, so on his own initiative he took all the capsules once a day. When he admitted to this practice, it was decided to continue 40 mg once a day, since when there have been no laboratory abnormalities and he remains free of symptoms.

Case 2

J.W., male, aged 14 years. Soon after birth he developed an erythematous skin and increasing scaliness worsening with time to produce a final clinical picture of generalized hyperkeratosis with a ridging pattern of his body folds and thick dark-brown scales on his trunk and limbs. A typical histological picture confirmed the diagnosis of congenital ichthyosiform erythroderma. At the age of 7 years he was given a 2-months' trial treatment with topical retinoic acid, 0·1% in a lanolin–vaseline base, with considerable improvement. Because of the severity of his generalized skin disease, he was commenced on oral retinoic acid, 10 mg four times a day, with gradual improvement over several months. The improvement was initially dramatic, but reached a clinical plateau which further improved with the 40 mg in one capsule a day. He had never been so free of scales before this therapy. He has no complaints and routine laboratory studies have always been normal. Attempts are now being made to reduce the maintenance dose.

Case 3

E.H., female, aged 11 years. Soon after birth she developed erythema and increasing scaliness of the skin which became a dark brown colour, most severe in body folds and on the neck. She had been diagnosed as suffering from congenital ichthyosiform erythroderma. She had had numerous salves in the past and a short trial with topical retinoic acid 0·1% in vaseline produced no change. She was commenced on oral retinoic acid one year ago, initially 10 mg a day and finally 20 mg twice a day. After 6 weeks she had a remarkable response and by 5 months was completely scale-free, but she has retained the typical reduced suppleness of her skin. Regular blood controls have been normal. She did notice mild cheilitis for a few weeks. It appeared not to be possible to diminish the maintenance doses in this young patient below 30 mg a day.

Case 4

M.B., female, aged 10 years. Soon after birth she developed an erythematous, fine scaling skin which worsened to a very severe generalized skin abnormality, characterized by markedly prominent ridging of body folds and spontaneous large serous blisters which readily ruptured to leave large erosions scattered randomly over her body. Her palms and soles were free of disease. Congenital bullous ichthyosiform erythroderma was diagnosed from the clinical picture and a skin histology of hyperkeratosis, acanthosis and intracellular oedema producing blisters in the upper part of the stratum Malpighii. No other physical abnormalities have been found. In 1971, retinol 200,000 units a day, was given for a few weeks with marginal improvement. It was stopped because of headaches. In the last year retinoic acid 10 mg four times a day was given orally with rapid clearing of the gross hyperkeratosis. The response continued with disappearance of the long-standing pruritis and of the blisters which had plagued her all her life. Although the clinical picture after 5 months is still not optimal, the patient now can appreciate a life free from constant skin sensitivity, painful erosions, secondary skin infections and the social embarrasment of strong body odour. We have raised her dose to 50 mg in one capsule a day without any problems.

DISCUSSION

Retinoic acid is an active end product of retinol metabolism. The intermediate metabolite, retinal, is essential for rhodopsin formation in the eye. This is a site where retinoic acid cannot participate physiologically, so damage to this organ can be avoided. Eye complications caused by excess of retinol were frequently limiting factors in treating potentially responsive dermatoses. The dosage regimen of retinoic acid was initially 10 mg a day in capsules. This was increased to 10 mg, four times a day, when there were no symptoms and regular liver function studies and blood counts were normal. No patients experienced untoward effects. Response to therapy was rather slow and unpredictable but all did improve. The dose has to be tailored for the patient, reducing it till keratin formation is just suppressed. When the total daily dose was given in one capsule there was further improvement without side effects. The therapeutic threshold for retinoic acid is not known so the optimal dosage programme still has to be determined. It has been found in rat experiments that retinoic acid is rapidly excreted from the body in the bile. Precisely what happens in man with this drug is not known. Blood levels of retinol, and carotene have been determined before and after retinoic acid administration. It was found in some dyskeratotic skin diseases that patients had below normal levels which increased after retinoic acid was given (Gunter, 1973). The significance of these findings is still speculative as retinoic acid cannot be converted into the premetabolites which were measured in this experiment. What is needed is an extensive investigation into the daily fluctuations of serum retinoic acid, and into the response to exogenous retinoic acid in normal and diseased people before an ideal therapeutic programme will be possible. Retinol has had a well established place in the therapeutic armamentarium for the dermatologist but it is becoming realized that one of its derivatives, retinoic acid, is supplanting it as it has a more specific effect on the skin and is less toxic.

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Case Reports

A case of variegate porphyria in an Indian

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SUMMARY

A female patient aged 25 years developed abdominal and neurological symptoms followed by a photosensitive eruption during pregnancy. She was found to have variegate porphyria on investigation. Investigation of the family revealed one member having latent porphyria. To our knowledge, this is the first report of variegate porphyria from India.

Dean & Barnes (1959) suggested the term variegate porphyria (VP) to describe a hereditary form of hepatic porphyria occurring in white South Africans. This form usually embraces photo-cutaneous, abdominal and nervous manifestations. The latter may or may not be concurrent with photosensitivity. Family relatives may have latent porphyria, acute systemic symptoms as in intermittent porphyria (AIP), or cutaneous as in porphyria cutanea tarda (PCT). In the present communication we report a female patient with VP, and one of her family members with latent porphyria, as the first cases in India.

CASE REPORT

A house-wife aged 25 years was hospitalized on 14 April 1972 with a recurrent bullous eruption of 5 years' duration and dark coloured urine for the last 3 months. The rash started during her first pregnancy 5 years ago. She had bullous lesions mostly confined to the light exposed areas, i.e. face, dorsal surfaces of the hands and feet, extensor aspect of forearms and lower legs. The lesions were exacerbated on exposure to sunlight. Relapses and short intervals of partial remission occurred. The cutaneous manifestations again worsened during her second pregnancy 3 years ago. The primary bullous lesions crusted on rupturing and some of the lesions healed to leave scars. There was a history of shedding nails.

About 1½ months before admission she developed non-radiating, mild, continuous, epigastric pain which lasted for the whole day, accompanied by nausea and vomiting. She was afebrile. A similar attack of abdominal pain occurred during her stay in hospital. She had been frequently constipated in the last 5–6 months.

About a month earlier she had had an episode of hysterical fits of unconsciousness with mild convulsions lasting for 6–7 h. She was given sedatives, including barbiturates, which seemed to have worsened her cutaneous state, and thereafter she was hospitalized.

Past history. She had always been in good health. Her two pregnancies were without obstetric complications. Menstruation was normal.

Family history. Her father died over 20 years ago. Her paternal uncle, who later married her mother, was symptom free but was found to have latent porphyria (*vide infra*). Her mother, husband and 2-year-old son are alive and healthy. Her 4-year-old daughter has had generalized bullous lesions almost since birth and on examination had multiple scattered impetigenized lesions without evident photosensitivity. The propositus has no siblings.

On examination. She had a moderate anaemia. Her liver was enlarged 2 cm below the costal margin, non-tender, soft. There was no splenomegaly. There were no neurological signs except some lower limb motor weakness. Cardiovascular and respiratory systems showed no abnormality; BP 160/100 mmHg during acute attacks, returned to 130/90 during remission. Her retinae were normal.

A bilaterally symmetrical vesiculo-bullous eruption with few shallow ulcers and crusts was present on the dorsal aspects of the hands and feet, extensor forearms and lower legs and face. There were some healed lesions with tissue-paper-like scars and warty lesions present on the dorsa of the hands. Involved areas were hypermelanotic, and there was hypertrichosis of the face. Her skin was fragile and Nikolsky's sign positive. Her nails were partially dystrophic, her teeth normal.

Diagnosis. The presumptive diagnosis of variegate porphyria was made clinically on the combination of symptoms resembling AIP and those of cutaneous photosensitivity.

Laboratory data. Haemoglobin 8 g/100 ml; white blood cell count 7500/mm^3; erythrocyte count 4·5 million/mm^3; ESR 25 mm, Westergren. Blood urea nitrogen, VDRL and Kahn tests, total and differential serum proteins, liver function tests, fasting and post-prandial blood sugar, ECG and chest X-ray were all normal. The serum cholesterol was 225 mg/100 ml.

TABLE 1. Urinary porphyrins and porphobilinogen (μg/24 h)

Type of porphyrin	Normal range	Present case
Coproporphyrin	1–283	957*
Uroporphyrin	0–40	35·9
Porphobilinogen	0–1000	+ screening test only*

* Indicates approx. significant abnormality.

TABLE 2. Faecal porphyrins (μg/g dry weight)

Type of porphyrin	Normal range	Present case
Coproporphyrin	0–50	90–70*
Protoporphyrin	0–113	324·0*
'Uroporphyrin'†	Nil	82·5*
X-porphyrin	0–8	8·4

† Ether insoluble, presumed uroporphyrin.
* Indicates significant abnormality.

TABLE 3. Red cell porphyrins (μg/100 ml cells)

Type of porphyrin	Normal range	Present case
Coproporphyrin	0–4·2	4·39
Protoporphyrin	0–37	13·2
Uroporphyrin	Traces or nil	Nil

Porphyrin studies

Qualitative. (1) *Urine.* (a) Dark brown in colour, qualitative screening tests for porphyrins highly positive (Rimington, 1961); (b) Watson–Schwartz test for porphobilinogen (PBG) positive.

(2) *Stools.* (a) Direct examination of stool specimen with Wood's lamp showed reddish fluorescence; (b) examination of the gloved finger after rectal examination gave red fluorescence.

(3) *Red cell microscopy.* No fluorocytes.

Quantitative. The results are shown in Tables 1, 2 and 3. Normal values of porphyrins are taken from Eales (1963) and Rimington, Lockwood & Belcher (1968).

Histopathologically, a skin biopsy of an intact vesicle from the dorsum of the hand showed a subepidermal bulla consistent with porphyria.

Investigation of patient's relatives

Uncle: Stools, qualitative screening test positive for porphyrin. Urine, qualitative test for urinary porphyrins negative. Watson–Schwartz test for PBG negative.

Other relatives. Qualitative estimation of urinary and faecal porphyrins and urinary PBG in mother, husband and two children negative.

Treatment

The patient was advised against taking barbiturates, sulphonamides, alcohol, chloroquin, griseofulvin, chlorpromazine, methylodopa, and oestrogens including contraceptive pills (Dean, 1965; Baxter & Permowicz, 1967), and to avoid sunlight and trauma. No other specific therapy was given.

DISCUSSION

This patient's case, the first reported from India, would seem to illustrate many of the features of VP. These include, (a) inheritance as a Mendelian dominant, (b) latent disease in relatives, (c) precipitation of acute systemic manifestations by barbiturates, (d) exacerbation by pregnancy (an inconstant feature). The absence of a raised faecal X-porphyrin may not be in accordance with the findings of Rimington *et al.* (1968) and of Magnus & Wood (1971), but may be due to the fact that the assay was done when the patient's disease was in remission.

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Panniculitis (Rothmann–Makai), with good response to tetracycline

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SUMMARY

A case of idiopathic circumscribed panniculitis of the legs, with features consistent with that of the Rothmann–Makai syndrome, is reported. The response to a 10-week course of oral tetracycline therapy was impressive. It is suggested that the anti-lipase activity of tetracycline could account for this response.

In 1964, in their review of 'lipogranulomatosis subcutanea' (Rothmann–Makai), Laymond & Peterson stated that 'There is no effective treatment'. No advance has been made in the therapy of the condition since (Cairns, 1972).

A patient with idiopathic circumscribed panniculitis (? Rothmann–Makai syndrome), showing good response to tetracycline therapy, is presented here. Although the condition is known to undergo spontaneous involution, the improvement, on exhibition of tetracycline, was so impressive that it is felt the response was truely a drug-related one. There is, moreover, sound clinical and laboratory evidence to support the use of tetracycline in 'panniculitis'.

CASE REPORT

H.S.Y., a 21-year-old female Chinese, was first seen on 24 September 1973 for red tender lumps over both legs of 1½ years' duration. The nodules erupted initially on the right lower leg followed soon after by nodules over the left leg. New lesions appeared one or a few at a time. Each nodule lasted about 3 weeks to 3 months. Many of the nodules did not resolve completely and new ones coalesced with old ones to form large plaques. Healing of nodules would lead to discoloration and depression of the skin surface. There was no spontaneous pain and no ulceration. Abdominal pain, fever, and other systemic disturbance were absent. No history of local injections was obtained. She was treated by several general practitioners without improvement.

Clinical examination revealed a well nourished young woman in good general condition. She was afebrile. Local examination (Fig. 1) showed erythematous plaques, each about 10–15 cm across, over both lower legs, almost encircling the legs above the ankles. These were firm and mildly tender on palpation. A few nodules of varying ages were seen away from the main lesions. One atrophic (depressed) hyperpigmented lesion was seen on the lateral aspect of the left leg. No ulceration was seen.

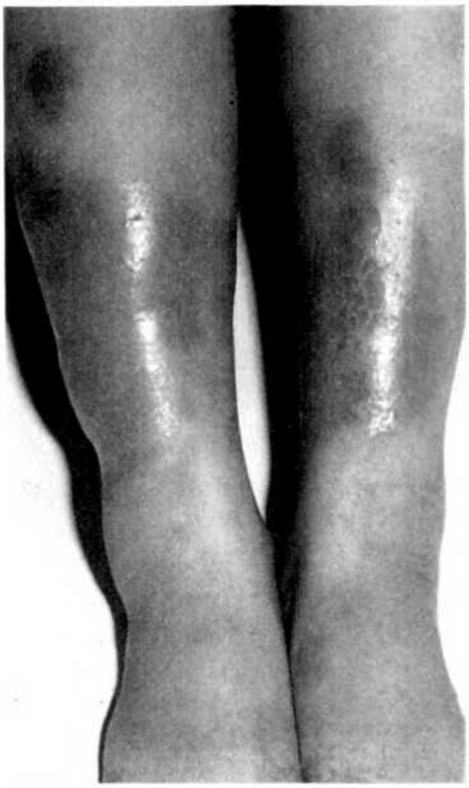

FIGURE 1. Large erythematous plaques over lower legs, before tetracycline therapy.

There were no lesions over the thighs, trunk, or face. The liver and spleen were not palpable. No clinical evidence of leprosy was detected.

Investigations

The haemoglobin was 10·7 g%; WBC count was normal; ESR was raised at 45 mm in 1 h. The blood urea, electrolytes, and liver function tests were normal. Serum amylase was 16 units (normal 0–16 units). Throat swab showed normal flora. Anti-streptolysin O titre was normal. Tests for L.E. cells and anti-nuclear factor were negative. Mantoux test was negative. Chest X-ray was normal. X-rays of the legs did not show any soft tissue calcification.

Skin biopsy (Fig. 2) showed an area of fat necrosis in the subcutis. Around the area of necrosis, an intense infiltrate was seen in between intact fat cells, consisting of histiocytes, lymphocytes, and a few giant cells. Some subcutaneous vessels showed mild endothelial proliferation. The epidermis and dermis were normal.

Treatment and Progress

She was started on oral tetracycline hydrochloride 250 mg three times a day. On review 2 weeks later, the patient had noticed that one of the leg nodules had spontaneously discharged an oily liquid. She was continued on tetracycline 250 mg twice a day for one month. Six weeks after starting therapy

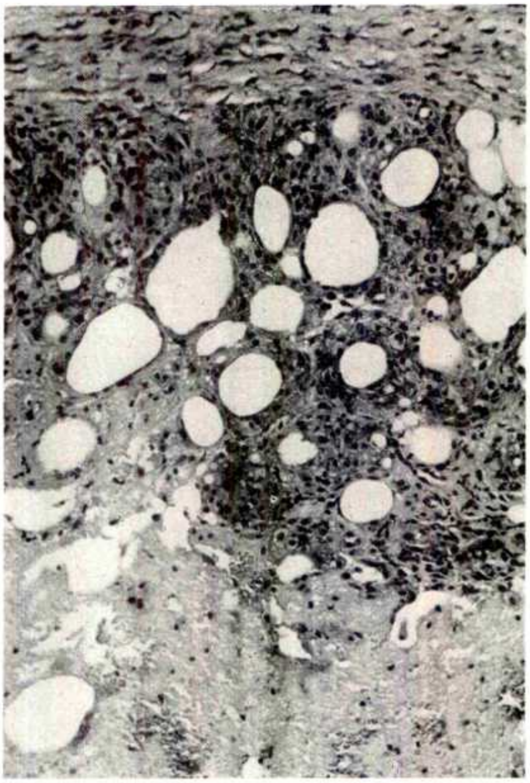

FIGURE 2. An area of fat necrosis in the subcutis, above which an intense infiltrate is seen in between intact fat cells. The infiltrate consists mainly of histiocytes and lymphocytes, a few giant cells were also seen (H & E, ×90).

the condition was much improved. The swelling and redness were subsiding and there were no fresh nodules. She continued oral tetracycline for a further month. Further resolution was seen 10 weeks after tetracycline therapy. The haemoglobin then was 12·8 g% and the ESR was 7 mm in 1 h.

DISCUSSION

Clinically there is very little doubt that the patient had a localized form of panniculitis. A hyperpigmented depression in the skin, following resolution of a nodose lesion, is a sign of panniculitis with fat necrosis. Biopsy confirmed the diagnosis. In erythema nodosum, necrosis of fatty tissue does not occur (Lever, 1967). The clinical picture is quite consistent with that described by Rothmann and Makai and reviewed by Laymond & Peterson (1964). In the Rothmann–Makai syndrome (1) new nodules do not occur in crops but rather one or only a few at a time; (2) the lower extremities are the sites most commonly affected; (3) there are no systemic symptoms; (4) although most cases occur in children, adult cases have been reported.

The histopathology of the above patient is also compatible with that described in cases of the Rothmann–Makai syndrome in the subacute stage.

The rationale of treatment of 'panniculitis' with tetracycline is based on the following evidence. In connection with work on acne vulgaris, tetracycline is known to alter the skin surface lipid composition. There is a decrease in the fatty acids and reciprocal increase in triglycerides (Freinkel *et al.*, 1965; Strauss & Pochi, 1966; Beveridge & Powell, 1969; Hassing, 1971). Hassing also showed that tetracycline can inhibit extrabacterial lipases. A recent investigation (Cunliffe *et al.*, 1973) demonstrated that oral tetracycline hydrochloride 250 mg a day caused a significant decrease in the fatty acid composition of the skin sebum, but no change in bacterial flora. They, therefore, suggested that the decreased fatty acid formation is due to a direct effect of the tetracycline on extracellular lipases. Inhibition of pancreatic lipase by tetracycline has also been shown (Shalita & Wheatley, 1970). There is, therefore, strong experimental evidence of the inhibitory influence of tetracycline, at therapeutic dosages, on body lipases.

Lipases are likely to play an important role in the pathogenesis of subcutaneous fat necrosis, and the subsequent inflammatory reaction of panniculitis, whatever the initiating factors. For example, it has been suggested that increased lipase activity is responsible for subcutaneous nodular fat necrosis in acute pancreatitis (Blauvelt, 1946). Serum lipase level was not estimated in this case because the laboratory procedure is difficult. It is also not known how serum lipase levels correlate with tissue lipase activity.

On theoretical grounds, therefore, improvement might be expected from treatment of 'panniculitis' with tetracycline, by preventing further lipolysis and fat necrosis. The case documented here did in fact show a very good response to tetracycline therapy.

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Comment

CHEMICAL ASPECTS OF HAIR LOSS AND ITS COSMETOLOGICAL SIGNIFICANCE

In a recent paper Kuchinka (1973) discussed some of the chemical aspects of hair loss. He reviewed a number of facts, findings and theories which might perhaps clear up the conflicting ideas regarding this phenomenon. Since Kuchinka's paper and the greater part of his references were published in the German language, they have been summarized in this Editorial.

If the weight of hair lost by combing (mg) is plotted versus the time following a shampoo (days), the amount lost invariably increases for about 2 weeks (Kuchinka, 1967). This loss may be considered to be non-pathological in nature. However, 24 hours after a second wash, the rate of hair loss returns to its original value. Thus, by continuing this process for a number of weeks, a characteristic saw-tooth graph is obtained. The implication is clearly that some component with a depilatory action is accumulating in the intervals between shampooings.

The depilatory property of hair lipids has been discussed frequently and has resulted in several interesting working hypotheses (Flesh & Goldstone, 1952; Hensecke & Neinass, 1957 and 1958; Hensecke & Schiefer, 1961; Weitzel, Fretzdorf & Heller, 1955). Analysis of the mixed hair lipids has shown the presence of large amounts of unsaturated fatty acids (Hougen, 1954; Nicolaides & Foster, 1956). It is difficult to believe that these are depilators *per se*, since they are neutral in their initial composition and presumably fulfil some normal biological function. They can, however, easily be auto-oxidised; this process accounts for the gradual increase in the odour of hair following a shampoo. It is now presumed that the increase in hair loss is also a consequence of oxidation products, a working hypothesis which has been supported by much experimental evidence.

The mechanism by which these oxidation products affect hair growth is still the subject of speculation. One possibility is that they alter the mitotic rate of the matrix cells. Another possibility is that they inhibit necessary enzyme systems in these cells; this is supported by the known sensitivity of many enzymes to peroxides *in vitro*. A third alternative is the direct interaction of lipoperoxides with prekeratin, with the result that normal keratinization cannot take place (Braun-Falco, 1960). Here there are several possibilities, the most likely being an inactivation of sulphydryl groups, preventing the important disulphide bridge formation in the keratin molecule. Experiments have confirmed that this can occur *in vitro*, and have also demonstrated the disruption of existing disulphide bonds (Duboulоz & Fondarai, 1953). In summary, its seems plausible that abnormalities of keratin biosynthesis might be an origin of increased hair loss, whilst enzyme inhibition in the matrix cells could perhaps influence the formation of new hair.

It must be noted that the pattern of hair loss induced by systemic toxic agents ('telogen effluvium') is quite different to that described above. For example, the oral administration of lipoperoxides to animals results in all 'all-or-none' alopecia developing after an appreciable latent period, whereas local application of lipoxidase plus linoleic acid causes a relatively rapid and dose-dependent hair loss similar to that found in human subjects (Puig Muset *et al.*, 1960).

The chemistry of the auto-oxidation of hair lipids is presumably similar to that of lipids in general. The primary reaction (double bonding of activated methyl groups) (Rieche *et al.*, 1962) gives rise to free radicals; these are able to bind oxygen. The simplified reaction scheme, which is a chain reaction, can be represented as:

$$RH \rightarrow R. + H.$$
$$R. + O_2 \rightarrow ROO.$$
$$ROO. + RH \rightarrow ROOH + R.$$

Thus the initial oxidative step will not greatly influence the odour or the appearance of the hair. Following the secondary reaction, however, changes in fat structure will occur, accompanied by the formation of a number of fission products ('rancidity') (Täufel & Rothe, 1949). The amount of oxygen bound by the primary reaction can be measured; this is called the estimation peroxide number.

It will be clear that the auto-oxidation of lipids can only usefully be influenced by modifying the first reaction, either by pro- or anti-oxidants, or by 'synergists' (Kaufmann, 1961). The first of these groups, pro-oxidants, comprises all those substances which favour the formation of new radicals. These include coloured compounds (haemoglobin, chlorophyll, etc.), certain enzymes (lipoxidase) and self evidently, peroxides themselves. Heavy metals, such as Cu and Fe, and (under certain experimental conditions) even NaCl, can act as pro-oxidants (Eberhardt, 1966).

Conversely, auto-oxidation can be retarded by compounds which inhibit the formation of free radicals. Most of these substances are hydrogen donors, usually possessing an active hydroxyl group, such as phenols which can form quinones. Examples of such compounds of natural origin are the tocopherols, flavanols, oxychromans (quercetin and its derivatives) gossypol, etc. Many synthetic anti-oxidants are derivatives of phenol; quinhydrone and hydroquinone are the most active (Täufel, Kretzschmann & Franzke, 1960), whereas 1-oxy-2-tert-butyl-4-methoxybenzole (butyl-hydroxyanisol, BHT) is the best-known. The gallates, nordihydroguajaretic acid and a large number of aliphatic oxy-acids also belong to this group.

Certain aromatic amines, for example N:N-diisopropyl-p-phenylene diamine and its nitro-derivatives exhibit an anti-oxidative action although these products do not possess an active hydroxyl-group. Here the mechanism still cannot be satisfactorily explained (Kaufmann & Korthage, 1953; Baltes, 1954; Wachs, 1957). Another group of anti-oxidants are the reductants, which offer a more reactive substrate to oxygen than do the lipids themselves. These include hydrazide, elementary sulphur (Bertram and Wynia, 1952), sulphites, thiosulphates, dialkyl-β-β-thiodipropionate and hypophosphite. The last group in this extensive range of compounds comprises those which are able to absorb energy quanta (e.g. ultraviolet radiation) directly, thus reducing the initial formation of free radicals.

The most widely-used 'synergists' are inorganic derivatives of phosphoric acid, citric acid, anhydrocitric acid, ascorbic acid and a number of amino acids, e.g. methionine. These substances are able to inhibit the effects of the pro-oxidants. Thus by combining synergists with anti-oxidants it is possible to increase the effectiveness of the latter by a factor of two or more. Practical examples are the 'silicon-oils mixtures'. The chelation or complex binding of heavy metal ions effects an anti-oxidant action in the same way.

The remarkable number of naturally occurring compounds possessing anti-oxidant activity makes it clear that this function is of biological importance *in vivo*. Examples include ubiquinone, bilirubin bile salts, biogenic amines, many vitamins, steroids, amino acids, certain hormones, and some xanthines (Kaufmann, 1961; 1962a; 1962b; Kaufmann & Garloff, 1961a; 1961b; 1961c). Further support for this concept has been provided by detailed investigations of the tocopherols (Heimann & Petzold, 1957; Hensecke & Neinass, 1957). Although the anti-oxidative activity of these compounds is markedly dependent on variables such as temperature, pH and concentration, the optimum value in each case coincided with the corresponding physiological parameter.

In healthy epidermis, a small quantity of fatty acid peroxide is nevertheless normally present (Szakall, 1952). The fact that the anti-oxidant concentration is higher in neoplastic than in normal cells (Zhuravlev, Tarusow & Esokova, 1966), coupled with the well-known anti-oxidant activity of carcinogenic hydrocarbons, suggests the possibility that an abnormally low level of peroxides may favour neoplastic changes. Thus caution is perhaps indicated in the indiscriminate use of powerful anti-oxidants.

Returning, however, to hair, our data may now be summarized by the statement that a medically and cosmetically acceptable inhibitor of lipid auto-oxidation will be of value in reducing hair loss. At the moment we cannot use strong anti-oxidants, since their side-effects on the skin, the extent to which they penetrate the epidermis, and the possible systemic consequences are insufficiently explored. It is likely that the cosmetic industry will eventually provide a range of suitable preparations, which will probably consist of mixtures of several different compounds. There is a good chance that the cyclic hair loss can be smoothed out in the course of a year, reducing the telogenic rate to about 4%.

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British Journal of Dermatology (1975) **92,** 359.

Correspondence

LICHEN SCLEROSUS ET ATROPHICUS IN THE BANTU

SIR, After the publication of our article Lichen Sclerosus et Atrophicus in the Bantu (*British Journal of Dermatology*, **91,** 81), I have been informed that F.P.Scott and J.G.H.Lups described two cases of Lichen Sclerosus et Atrophicus affecting Bantu patients. Consequently the sentence in paragraph 2, page 82, should be changed to 'We therefore describe four cases of LSA, one of the most uncommon skin disorders in the Bantu (Scott & Lups, 1971).'

M.DOGLIOTTI

Department of Dermatology
Baragwanath Hospital
Johannesburg (S.A.)

REFERENCE

SCOTT, F.P. & LUPS, J.G.H. (1971) Lichen sclerosis et atrophicans by die Bantoe. *Geneeskunde*, **13,** 76.

EARLIEST DESCRIPTION OF THE ATOPIC SYNDROME?

SIR, The description of the Emperor Augustus given by the Roman historian Suetonius (born about A.D. 69) includes the following observations: 'His body is said to have been marred by . . . . a number of hard, dry patches suggesting ringworm, caused by an itching of his skin and a too vigorous use of the scraper at the baths'. Later the writer adds that Augustus was subject to 'certain seasonal disorders: In early spring a tightness of the diaphragm; and when the sirocco blew, catarrh'.

There seems little doubt that the combination of lichenified skin lesions, asthmatic symptoms and rhinitis indicates an atopic predisposition. Surely Suetonius's account (which seems to have been overlooked by modern dermatologists) must be the earliest recorded description of this syndrome?

P.D.MIER

Department of Dermatology,
(Professor Dr. J.W.H.Mali)
University of Nijmegen,
The Netherlands

REFERENCE

SUETONIUS (A.D. 69–140) The twelve Caesars (Trans. by Robert Graves), p. 95. Penguin Books Ltd., Harmondsworth, Middlesex.

TAKING *P* VALUES OUT OF SALES TALK

SIR, Drug reps (short for repetitive?) repeatedly bludgeon us with the information that double-blind controlled trials of their latest nostrum have shown it to be significantly superior to its rivals, with $P < 0{\cdot}05$. I suggest that before succumbing to statistricks we ignore the P value and examine the data. I read recently, for example, that in a trial of A and B in twenty-eight patients, A was superior in fifteen, B was superior in six and in seven no difference was detected. The claim for statistical significance was based on the fact that, leaving out the seven patients with an equal result, χ^2 was 3·86 with $P = 0{\cdot}05$. This erroneous use of the χ^2 test would have given the same level of significance if there had been 1028 patients in the trial, with A superior in fifteen, B superior in six, and no detectable difference in 1007.

This particular example occurred in the *British Journal of Dermatology*, but similar statistical solecisms can be found in most journals. As non-mathematicians we may hesitate to cast out the statistical mote, but where new drugs are concerned we can and should look at the basic data and refuse to be drowned in an eloquent flood of P values.

J.L.BURTON

Dermatology Department,
Bristol Royal Infirmary,
Bristol

British Journal of Dermatology (1975) **92**, 361

Book Review

Scleroderma (Progressive Systemic Sclerosis). A.J.BARNETT (1974) Springfield, Illinois. Charles C.Thomas. Pp. xiii+255, illustrated. Price £10.10.

In the past two decades there have been excellent monographs on this subject every few years (Orabona & Albano, 1958; Jablonska, 1965; Sackner, 1967). The author of this book combines a review of the literature to early 1973 with his personal experience of more than sixty cases seen in Australia over 20 years. He distinguishes morphoea from systemic sclerosis and classifies the latter into three groups on the basis of the type of skin involvement: Type 1, skin involvement remaining localized to the fingers; Type 2, skin involvement spreading slowly to involve hands, forearms and face; Type 3, skin involvement diffuse from the onset or rapidly progressive. These types are not absolutely rigid and all may have systemic involvement. Sixty per cent of patients with Types 1 and 2 usually survive more than 10 years whereas Type 3 have a much shorter survival time. He agrees that the so-called 'CRST syndrome' is not a separate entity.

There is a good simple account of the connective tissue disturbances in systemic sclerosis. The author tends to reject an auto-immune aetiology for the disease although he admits that cell-mediated immunity could be involved. There is still no satisfactory treatment.

Sensible commentaries are present throughout the text. This book can be recommended to all those who want an up-to-date account of this unpleasant, but fascinating, disease.

N.R.ROWELL

British Journal of Dermatology (1975) **92**, 362,

News and Notices

Brief Communications

In future this Journal will be prepared to consider 'brief communications' taking up less than one page of journal space. These papers will be listed in the Contents page and will therefore appear in the index and in abstracting journals. The papers will run on one after the other, as in the Correspondence and Editorial pages of the Journal, and will therefore take up less than the minimum of two pages necessary for original contributions.

Reprints will be available, but may well contain fragments of other authors' contributions.

It is hoped that these pages will be suitable for:

(a) Short papers meriting urgent publication.

(b) Results of investigations in which full details, for example, of techniques and discussion are unnecessary.

(c) Short case reports, some of which may only be black and white photographs with two or three lines of text.

Ninth Miles International Symposium

The Johns Hopkins Medical Institutions, Baltimore, Md., U.S.A. June 4–6, 1975

This Symposium concerns the identification and role of cellular receptor sites for a variety of biologically active entities, including viruses, antigens and antibodies, polypeptide hormones and small molecules. The participation of these interacting systems in immunological, endocrinological and pharmacological phenomena within the cells has become a subject of increasing interest and importance in biomedical research during the past 10 years.

For further information contact: Edward G.Bassett, Ph.D., Symposium Coordinator, Miles Laboratories, Inc., Elkhart, IN 46514 U.S.A., Tel. (219) 264–8460.

Three-day Dermatopathology Symposium

A three-day Dermatopathology Symposium entitled 'Histologic Diagnosis of Inflammatory Skin Diseases', sponsored by the departments of dermatology and pathology of New York University School of Medicine, will be held from 26–28 June 1975. Directed by Dr A.Bernard Ackerman, Associate Professor of Dermatology and Pathology, it will take place in Alumni Hall, NYU Medical Center, 550 First Avenue, New York, N.Y.

For further details inquire at the Office of the Associate Dean, New York University Post-Graduate Medical School, 550 First Avenue, New York, New York 10016. Tel. (212) 679-3200, Ext. 4037.

Dermatologic

Offizielles Organ der Schweizerischen Gesellschaft für Dermatologie und Venerologie
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Index Vol. 149, No. 2, 1974

Reymann, F. (Copenhagen): Treatment of Discoid Lupus Erythematosus with Betamesone–Valerate Cream 1%

MacKie, Rona M. (Glasgow): Sweet's Syndrome. A Further Case of Acute Febrile Neutrophilic Dermatosis

Remy, W.; Bockendahl, H., and Antoniadis, G. (Berlin): Comparative Studies of Rubella Antibodies in Psoriatic Patients Treated with Methotrexate

Pospíšil, L. and Kratochvil, F. (Brno): Vor- und Nachteile des Peroxidase-Tests mit *Treponema pallidum* in der Serodiagnostik von Syphilis im Vergleich mit dem FTA-ABS-Test
(Advantages and Disadvantages of the Peroxidase Test with *Treponema pallidum* in Diagnostics of Syphilis as Compared with the FTA-ABS Test)

Hagedorn, M.; Thomas, C. und Kannen, W. von (Freiburg i. Br.): Dermatofibrosarcoma protuberans mit Übergang in ein sogenanntes Fibrosarkom
(Dermatofibrosarcoma Protuberans with Transition into a Socalled Fibrosarcoma)

Björnberg, A. (Gothenburg): Low Alkali Resistance and Slow Alkali Neutralization. Characteristics of the Eczematous Subject?

Bruyn, C.H.M.M. de and Oei, T.L. (Nijmegen): Purine Phosphoribosyl Transferases in Human Hair Roots

Undeutsch, W. und Schieferstein, G. (Tübingen): Das Knoblauchzehenfibrom
(The Garlic-Clove Fibroma)

Borovanský, J. and Duchoň, J. (Prague): Chemical Composition of Hair Melanosomes

Christiansen, J.V.; Gadborg, E.; Ludvigsen, K.; Konstmann Meier, C.H.; Nørholm, A.; Osmundsen, P.E.; Pedersen, D.; Rasmussen, K.A.; Reiter, H.; Reymann, F.; Rosman, N.; Sylvest, B.; Unna, P.; Wehnert, R.; Aastrup, B.; Andersen, B., and Holm, P. (Aarhus/Copenhagen): Topical Vitamin A Acid (Airol®) and Systemic Oxytetracycline in the Treatment of Acne Vulgaris. A Controlled Clinical Trial

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Textbook of Dermatology

Edited by

Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and
F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

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Edited by J. M. Blakeway

VOL. 25 NO. 7 JULY 1974

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T. J. ELLIOT. Use of a laboratory model to evaluate the factors influencing the performance of depilatories.

MEDAL LECTURE

F. J. EBLING. Sex hormones and skin.

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microgram(s) (10^{-6} g)	µg	cubic millimetre(s)	mm^3	micrometer(s)	µm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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British Journal of Dermatology, Volume 92, Number 3, March 1975

CONTENTS

PAGE

Clinical and Laboratory Investigations

Micro-determination of Cyclic AMP Levels in Human Epidermis, Dermis and Hair Follicles 241
K. YOSHIKAWA, K. ADACHI, V. LEVINE AND K. M. HALPRIN

Cyclic AMP in Skin: Effects of Acute Ischaemia 249
K. YOSHIKAWA, K. ADACHI, K. M. HALPRIN AND V. LEVINE

Further Studies on Adenyl Cyclase in Psoriasis 255
MEI MEI MUI, S. L. HSIA AND KENNETH M. HALPRIN

Dermal–Epidermal Deposition of Complement Components and Properdin in Systemic Lupus Erythematosus 263
ROBERT E. JORDON, ARNOLD L. SCHROETER AND R. K. WINKELMANN

Tumour Angiogenic Factor Associated with Subcutaneous Lymphoma 273
JOHN E. WOLF AND WINTHROPE R. HUBLER, JR.

Psoriasiform Napkin Dermatitis—A Follow-up Study 279
E. A. NEVILLE AND O. A. FINN

Nickel Dermatitis Hazards from Prostheses—*In Vivo* and *In Vitro* Solubilization Studies 287
M. H. SAMITZ AND S. A. KATZ

Growth Inhibition in Normal Mammalian Melanocytes *In Vitro* 291
P. A. RILEY

Primary Cell Culture for Biochemical Studies of Human Keratinocytes—A Method for Production of Very Large Numbers of Cells Without the Necessity of Subculturing Techniques 305
B. ALLEN FLAXMAN AND ROBERT A. HARPER

The Junctional Zone Beneath Migrating Epidermis 311
R. MARKS, E. ABELL AND T. NISHIKAWA

The Removal and Restitution of Hair Fat in the Rat 321
F. J. EBLING AND J. SKINNER

The Sebotrophic Action of Growth Hormone (BGH) in the Rat 325
F. J. EBLING, ERIKA EBLING, VALERIE RANDALL AND J. SKINNER

Mites in the Environment of Cases of Norwegian Scabies 333
R. WORKMAN CARSLAW, RONALD M. DOBSON, A. J. K. HOOD AND R. N. TAYLOR

Pharmacology and Treatment

High Dose Ascorbic Acid in the Management of Thalassaemia Leg Ulcers—A Pilot Study 339
A. M. AFIFI, L. ELLIS, R. G. HUNTSMAN AND M. I. SAID

Oral Retinoic Acid as Therapy for Congenital Ichthyósiform Erythroderma 343
L. ERIKSEN AND R. H. CORMANE

Case Reports

A Case of Variegate Porphyria in an Indian 347
F. HANDA, KAMLESH KUMAR AND RAJ KUMAR

Panniculitis (Rothmann–Makai), with Good Response to Tetracycline 351
H. L. CHAN

Comment

Chemical Aspects of Hair Loss and its Cosmetological Significance 355
F. A. J. THIELE

Correspondence

Lichen Sclerosus et Atrophicus in the Bantu 359
M. DOGLIOTTI

Earliest Description of the Atopic Syndrome 359
P. D. MIER

Taking *P* values Out of Sales Talk 359
J. L. BURTON

Book Review 361

News and Notices 362

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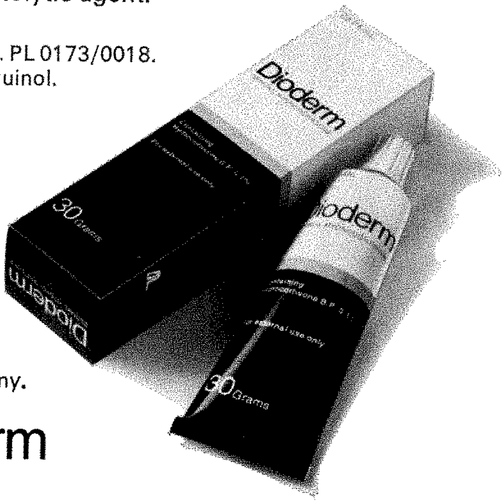

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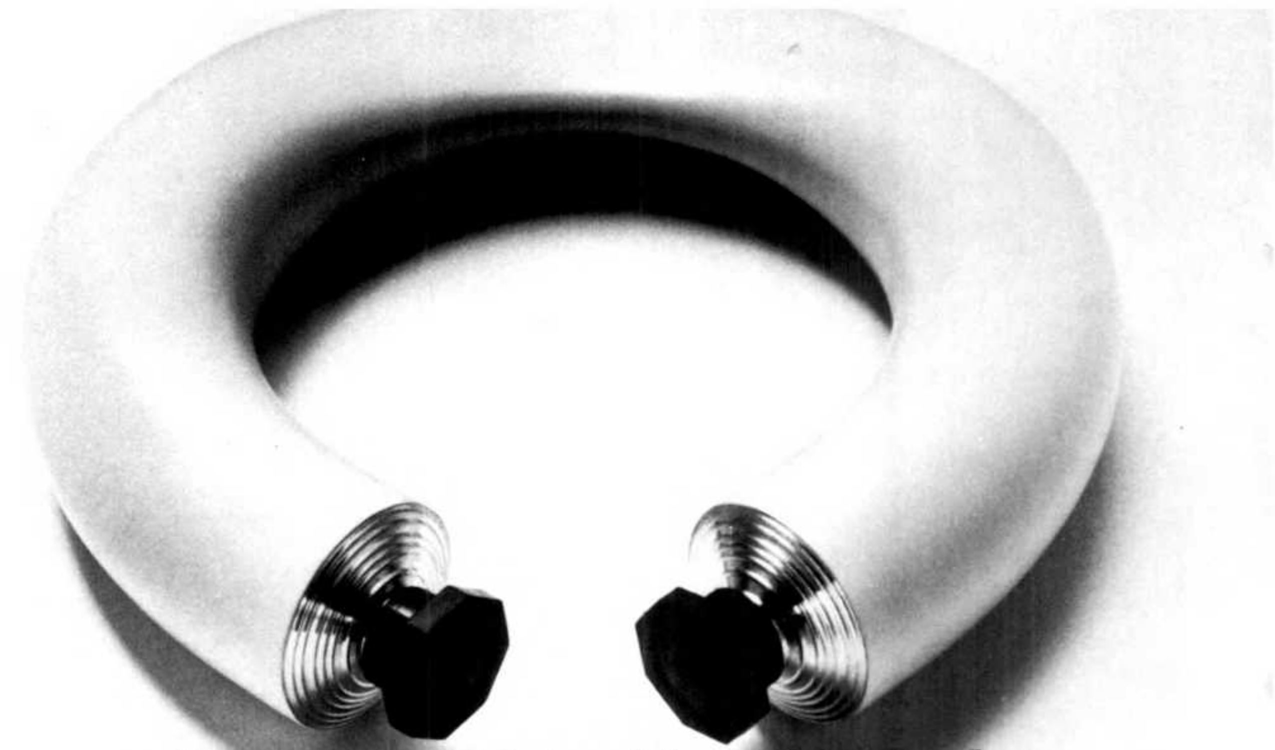

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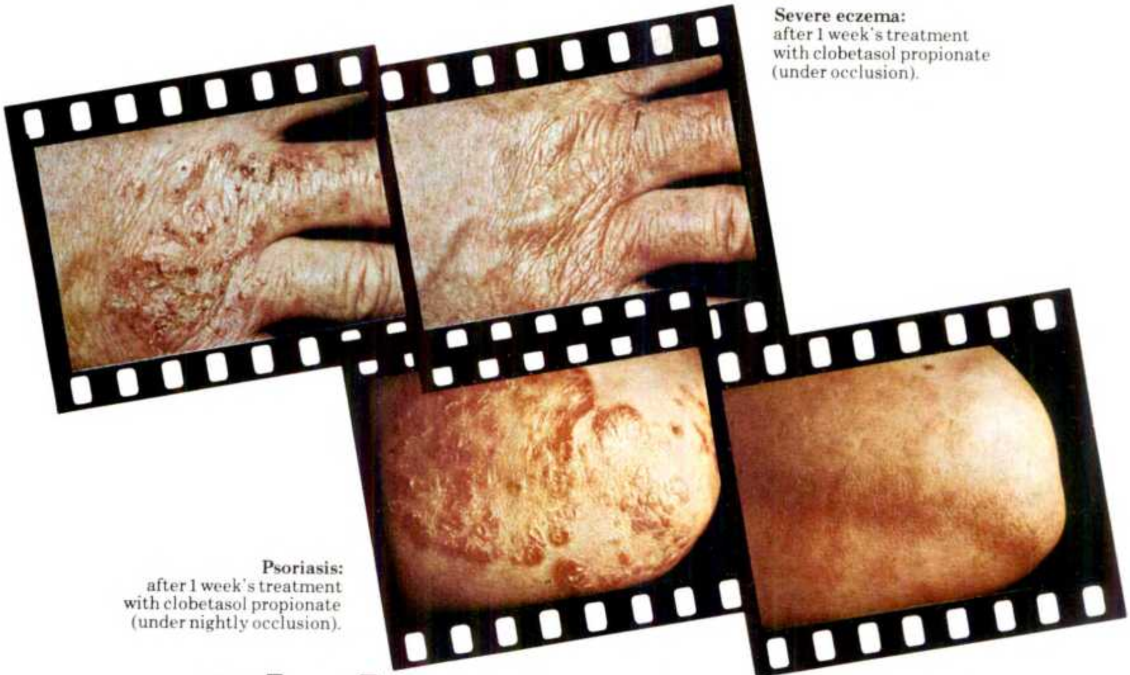

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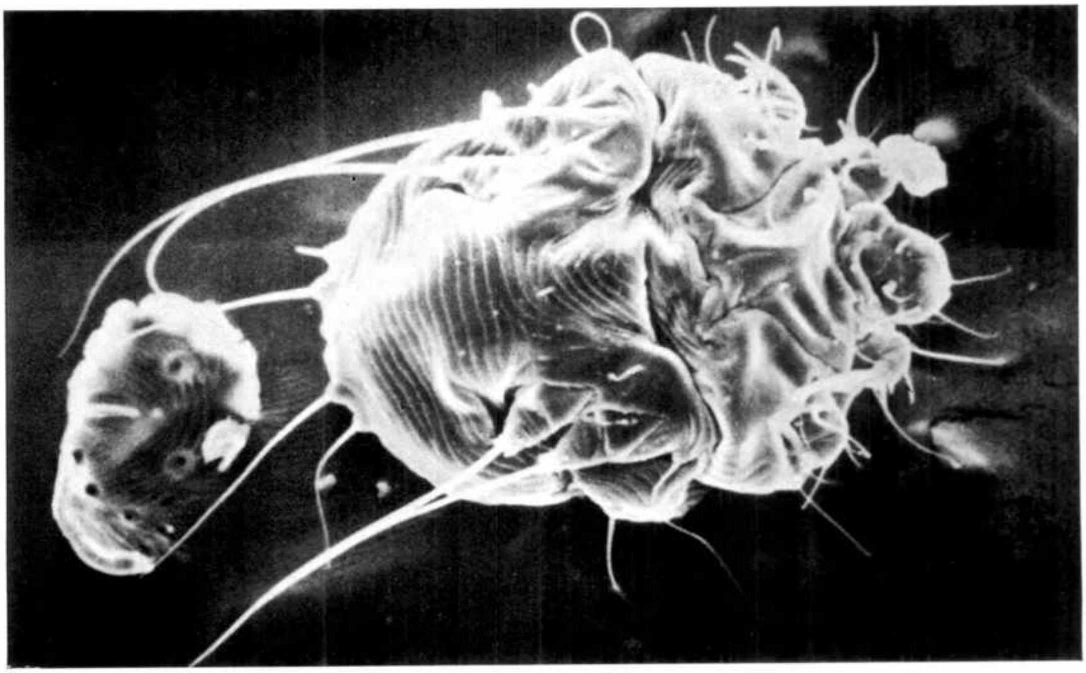

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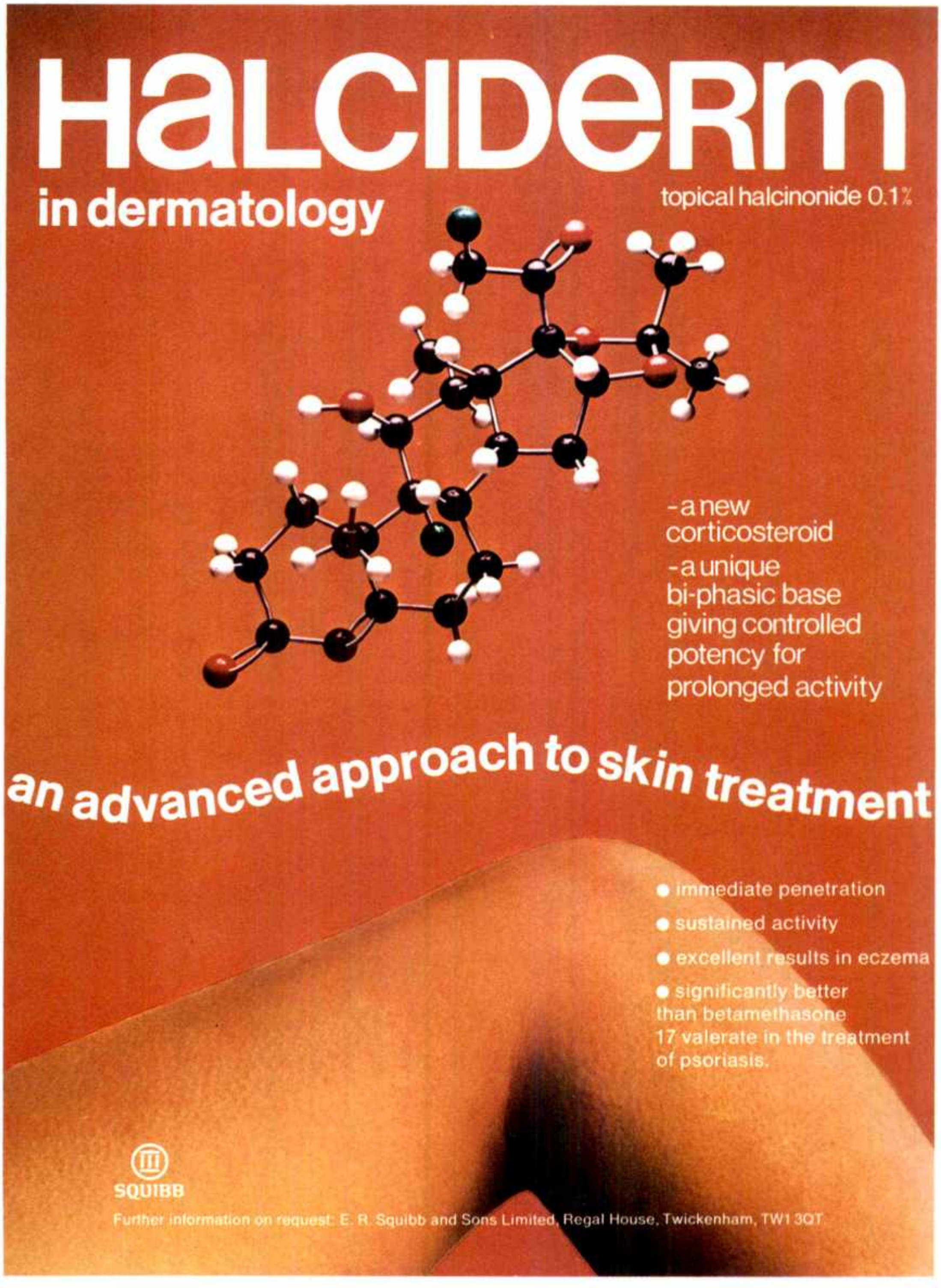

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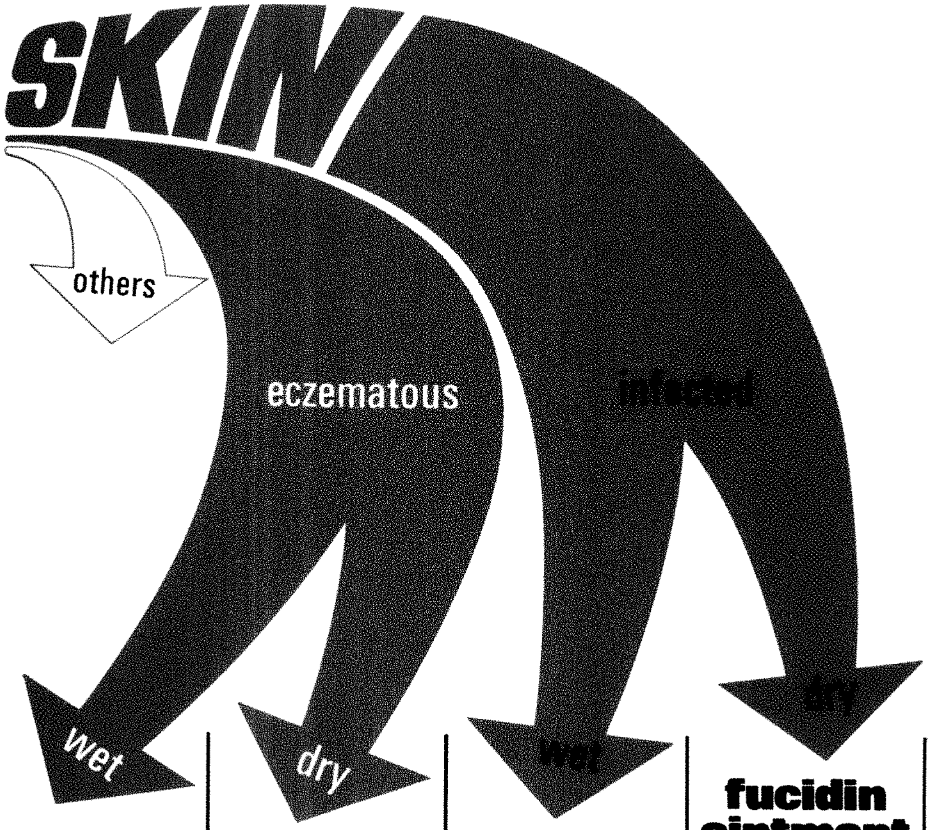

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Index Vol. 149, No. 1, 1974

Polano, M.K. (Leiden): Xanthomatosis and Hyperlipoproteinemia. A Review

Guerrier, C.J.W. and Wong, C.K. (Paris/Oxford): Ultrastructural Evolution of the Skin in Incontinentia pigmenti (Bloch-Sulzberger). Study of Six Cases

Weirich, E.G. und Longauer, J. (Basel): Topographische Unterschiede in der Ausbildung und Reaktivität der Aurikulartalgdrüsen des Kaninchens. II. Mitteilung. Histometrische Untersuchungen zur Talgdrüsenaktivität
(Topographical Differences in the Development and Reactivity of the Auricular Sebaceous Glands of the Rabbit. II. Communication: Histometric Studies of the Activity of the Sebaceous Glands)

Hägermark, Ö.; Wahlberg, J.E., and Germanis, M. (Stockholm): Determination of Oxyquinoline Concentrations in Plasma in a Patient Treated for Acrodermatitis enteropathica – an Aid in Therapeutic Control

Hellgren, L. and Vincent, J. (Göteborg): Skin Surface Retention of an Applied Ointment

Bhutani, L.K.; Bedi, T.R.; Pandhi, R.K., and Nayak, N.C. (New Delhi): Lichen planus pigmentosus

Clayton, R. and Wood, P. (London): Subcutaneous Nodular Sarcoid

The Interesting Case · Der interessante Fall · Un cas intéressant

Hopf, B. und Krebs, A. (Bern): 'Ulcera cruris' als seltene Form einer Sarkoidose
('Ulcera cruris' as a rare manifestation of sarcoidosis)

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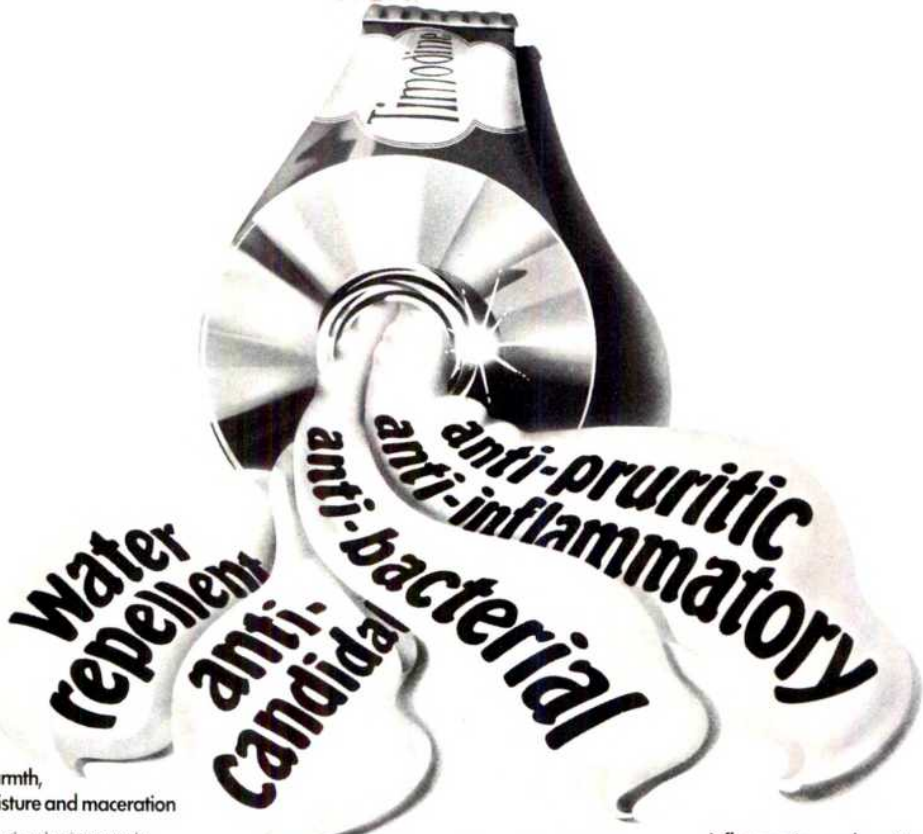

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1. Almeyda, J. and Burt, B.W., *Brit. J. Derm.* (To be published) 2. Harries, E. H. L. and Jacoby, R. H. (To be published)

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British Journal of Dermatology (1975) **92**, 363.

Clinical and Laboratory Investigations

The clinical spectrum of Sweet's syndrome (acute febrile neutrophilic dermatosis)—a report of eighteen cases

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SUMMARY

Eighteen cases of Sweet's syndrome are described. This is the largest series so far reported and the first from the tropics. The essential features are the characteristic morphology, the histological appearances, the dramatic response to corticosteroids and the absence of scarring. Attention is drawn to the frequent involvement of the eyes and joints during the course of the illness. Fever and neutrophilia were found less frequently than a raised ESR or a preceding upper respiratory tract or skin infection. The term Sweet's syndrome is preferred to acute febrile neutrophilic dermatosis. The frequency of occurrence of different clinical manifestations is discussed.

In 1964, Sweet described a 'distinctive and a fairly severe illness' of obscure aetiology which he had observed in eight patients between 1949 and 1963, under the title 'acute febrile neutrophilic dermatosis'. The cardinal features were, 'fever, neutrophil polymorphonuclear leukocytosis of the blood, raised painful plaques of the limbs, face and neck, and histologically a dense dermal infiltration with mature neutrophil polymorphs'. The other important features were the absence of any evidence of infection, the dramatic response to corticosteroids and the absence of scarring.

A patient with a clinical syndrome apparently identical with that described by Sweet was reported by Robinson in 1965. Fuld (1965) recounted three cases with a similar clinical picture. Crow, Kerdal-Vegas & Rook (1969) described three further cases which were 'in every way indistinguishable from those reported by Sweet'. Although they found that the symptoms and signs showed a considerable variation in severity and duration, they were impressed by 'the consistency and the individuality of the (essential) clinical and histological features', which perhaps prompted them to use the eponym Sweet's syndrome for this condition. The typical skin eruption they described as follows: 'The skin lesions are initially dull red plaques or nodules, dermal or hypodermal. They regularly involve the forearm, and often the back of the hand; the face, neck and the shins are involved only slightly less frequently. In a few cases the lesions have developed on the shoulders, the thighs or the abdomen.

Reprint requests to Dr D.A.Gunawardena, General Hospital, Kandy, Sri-Lanka

The lesions are usually tender and sometimes spontaneously painful. Without treatment new lesions continue to appear more or less symmetrically for several weeks. Some lesions enlarge slowly for 7–10 days, then slowly resolve to leave some pigmentation. The majority, however, enlarge slowly and irregularly and soon resemble a miniature relief model of a mountain range with pustules, always sterile, surmounting the peaks. Partial central clearing may accentuate the deeper red and more actively pustular, advancing marginal mountain range surrounding the central fading erythema and pigmentation'. They also drew attention to preceding infections.

Sweet (1968) reported a further five cases which differed somewhat from his original description, in that some had neither fever nor leukocytosis. However, he was able to diagnose the condition 'at a glance' by the characteristic morphology of the lesions. His fifth patient had a lesion confined to the face. This report was followed by that of Whittle, Beck & Champion (1968), who described two cases in whom the lesions were localized to the face. These they considered to be variants of Sweet's syndrome. One of them was the first case report in a male. Three other male patients were reported by Brenan (1969), Shapiro, Baraf & Richheimer (1971), and Goldman & Moschella (1971). Three further cases were reported by Evans & Evans (1971) and Frayha, Matta & Kurban (1972), making a total of twenty-five cases reported to date.

We have seen eighteen patients with this syndrome between July 1971 and March 1973. This series is the largest so far published and the first from the tropics. Like Crow *et al.* we are impressed by the consistency and the individuality of the essential clinical features comprising this syndrome, and the ease of diagnosis with increasing awareness of the condition. We have in addition noticed frequent involvement of the eyes and joints during the illness.

CASE REPORTS

The eighteen patients were seen between July 1971 and March 1973 from among 4570 new patients treated at the Dermatological Unit of the General Hospital, Kandy, Sri-Lanka. This is the only dermatological unit in the area serving a population of about 1·7 million, and tends to see the more severe dermatoses. All the patients in this series were referred by Medical Practitioners to the senior author (D.A.G.) for dermatological opinion. Scalpel biopsies of representative lesions were done on all patients on at least one occasion. All but two patients were treated as in-patients during the acute phase of the illness. They were observed over a period varying from 11 days to 19 months.

Four illustrative case reports are followed by a summary of the clinical and histological features of the eighteen patients.

Case 1

In this patient serial biopsies were taken.

A 47-year-old female had a secondary vaccination against smallpox on 8 February 1972. On 11 February she developed fever with itching, redness and swelling around the vaccination site on the left forearm. On 15 February an eruption appeared on the right upper arm and the back of the chest. On 18 February she developed 'red eyes' and was then referred to us with a provisional diagnosis of an 'allergic reaction to vaccination'.

When seen on 21 February she was apyrexial, and had an echthymatous lesion on the left forearm with surrounding cellulitis. The right upper arm and the back of the chest showed dusky red, raised, indurated plaques with partial central clearing. She also had bilateral conjunctivitis.

Her ESR (Westergren) was 35 mm/h and WBC 6000/mm^3 (72% polymorphs, 22% lymphocytes, 6% eosinophils). Urinalysis was normal. A biopsy from the right upper arm on 22 February showed no histological evidence of vaccinia. The epidermis showed moderate spongiosis. In the dermis there was moderate interstitial oedema, most prominent in the mid and upper dermis especially around blood vessels. There was a striking perivascular inflammatory reaction confined mainly to the upper and mid dermis (Fig. 1a, b). In focal areas this reaction appeared to affect the plexuses of small vessels, consisting of polymorphs (neutrophils and eosinophils), a smaller proportion of lymphocytes and an occasional histiocyte. Intact and disintegrated red cells and an occasional haemosiderin laden macrophage were seen in the infiltrate. In other areas, the infiltrate was in close proximity to the vessel wall without much extension beyond it. Here the predominant cells were lymphocytes.

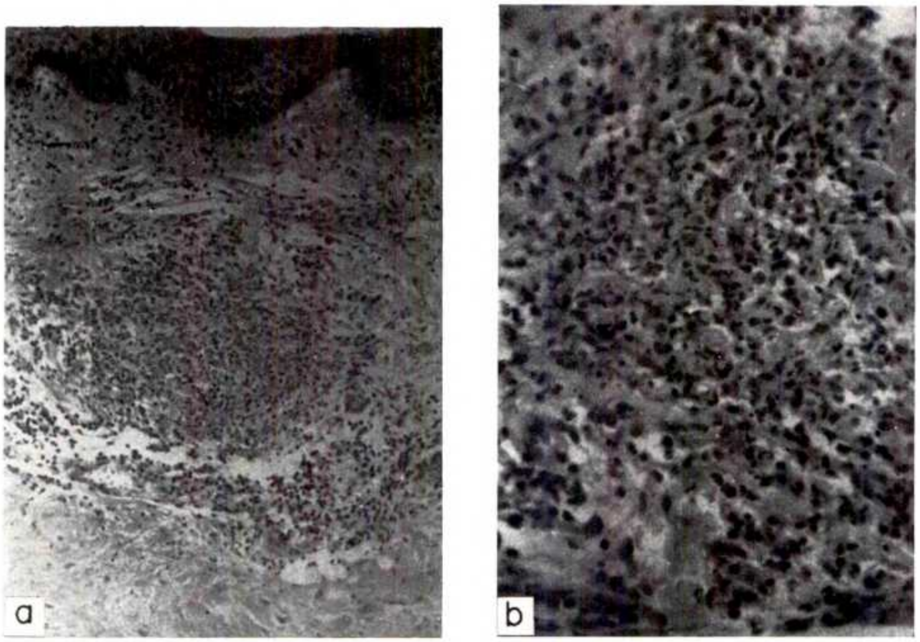

FIGURE 1. Case 1. (a) A week old lesion (22 February 1972) showing dermal oedema and a focal infiltrate (× 50); (b) High power view of the focal area showing the predominantly neutrophilic exudate (× 200).

The vaccination wound was treated with wet dressings, and when she was discharged on 29 February this wound had healed, the other skin lesions were flatter and the eyes were less red. She was not given steroids at any stage of her illness.

A second biopsy done before discharge, close to the first biopsy site, showed moderate hyperkeratosis of the epidermis, mild dermal oedema with a prominent perivascular infiltrate in the upper and mid dermis. The infiltrate consisted mostly of lymphocytes and histiocytes. There were no polymorphs or red cells.

By 2 March the skin lesions were flatter. The right eye still showed a flame shaped redness on the lateral side. A third biopsy was done on 18 March, close to the earlier sites. The epidermis was histologically normal. The infiltrate was focal and less extensive than before (Fig. 1c), and consisted mostly of lymphocytes and a few histiocytes. An occasional fibroblast was seen. The vessels were undamaged. Her ESR at this stage was 5 mm/h. A fourth biopsy was done on 30 March, close to the previous biopsy sites. The epidermis was normal. There was a mild perivascular lymphocytic infiltrate in the mid and upper dermis. There was no fibrosis or permanent damage to the vessels.

When seen on 21 February 1973, she had had no recurrences of her cutaneous or ocular lesions and there was no scarring.

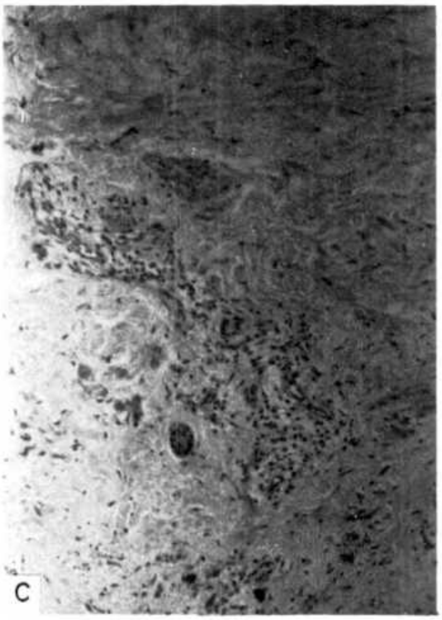

FIGURE 1. Case 1 (c) A month old lesion (18 March 1972) showing a localized perivascular infiltrate which is less extensive and is now predominantly lymphocytic (H&E, × 50).

Case 2

This case illustrates the development of ocular and joint symptoms during the illness.

A 43-year-old female developed a cough, cold, sore throat and fever on 16 April 1972. She developed a rash on her legs on 19 April, and 'red eyes' the next day. On 21 April she had pain and swelling of her left knee joint. She has had recurrent joint pains involving the hips, knee, shoulders, neck and spine for over 15 years. For the last 7 years she has had recurrent conjunctivitis. She was admitted to a medical ward of this hospital on 26 April, with a suspected diagnosis of erythema nodosum and was treated with aspirin and promethazine.

When seen by us on 28 April, she was afebrile and had bilateral episcleritis affecting the right eye more than the left. The left knee joint had an effusion and was tender. There were the characteristic red, raised, well defined plaques and nodules on the neck, front of the chest and legs.

Investigations revealed an ESR of 115 mm/h; a WBC of 9,600/mm^3 (80% polymorphs, 20% lymphocytes); Hb 9·4 gm/100 ml, P.C.V. 32%, M.C.H.C. 30%. The blood film was normal. Total serum proteins were 7·7 gm/100 ml (albumin 2·3 gm/100 ml, alpha$_1$ globulin 0·6 gm/100 ml, alpha$_2$ globulin 0·9 gm/100 ml, beta globulin 1·3 gm/100 ml and gamma globulin 2·6 gm/100 ml). Throat swab yielded coagulase positive *Staphylococcus aureus*. Urine and stools showed no abnormality. Blood urea was normal. Examination of blood for microfilaria and L.E. cells was negative. Chest X-ray was clear.

Biopsy of a 9-day-old lesion showed mild hyperkeratosis, with focal areas of acanthosis and moderate spongiosis of the epidermis. There was moderate oedema in the upper and mid dermis. The upper dermis showed a marked perivascular infiltrate which extended to the mid dermis around the adnexal blood vessels. In some focal areas where the infiltrate was intense the blood vessels showed endothelial swelling. In the centre of these focal areas was an infiltrate of dead and dying polymorphs, and some histiocytes and lymphocytes.

She was started on prednisolone 30 mg daily on 1 May and the dosage was reduced to 20 mg daily on 5 May. Her joint, eye and skin signs improved and the dosage was further reduced to 15 mg daily on 8 May. ESR was then 28 mm/h. She was discharged on this dosage on 9 May, but she did not return for follow up.

Case 3

This patient also developed ocular and joint symptoms during the course of the illness, which followed secondary vaccination against smallpox.

A 70-year-old female was vaccinated on 13 February 1972. She developed fever on 16 February which settled in about 2 days. On 20 February, she had fever with rigors, and a burning sensation in her extremities. This was followed by the appearance of an erythematous eruption on the palms and soles. She noticed 'red eyes' and pain in the neck on 23 February. She had had occasional pain in the neck and red eyes for over 2 years but the symptoms were worse.

On examination on 23 February she had a temperature of 38·3°C and a healing vaccination wound. She had bilateral episcleritis and the characteristic eruption of plaques and nodules on the left forearm, back of the chest, palms and soles.

Investigations showed an ESR of 58 mm/h; WBC of 13,600/mm^3 (53% neutrophils, 32% lymphocytes, 15% eosinophils). Total serum proteins were 8·1 gm/100 ml; albumin 3·8 gm/100 ml and globulin 4·3 gm/100 ml. Urine showed a trace of albumin and 10–12 pus cells per field. Hb 8·3 gm/100 ml, P.C.V. 26%, M.C.H.C. 32%. Chest X-ray was normal except for an upper dorsal scoliosis. V.D.R.L. was non-reactive.

A biopsy done on the 10th day of the eruption showed histological changes essentially similar to those of Case 2.

She was treated with aspirin, promethazine and ferrous sulphate and as her skin condition was improving she was discharged on 4 March. When seen again on 9 March, she had some pain in the neck. The episcleritis had cleared and her skin appeared normal, apart from some scaling of the palms and soles. When reviewed 1 year later there was no scarring and she has had no relapses.

Case 4

This case is of interest, because his skin lesions could have been mistaken for leprosy, and because he is the only male in our series.

A 61-year-old male was referred to us by a venereologist to exclude a diagnosis of leprosy. The patient had developed a cough on 7 September 1971. Soon after, he noticed a non-pruritic skin eruption which appeared first on his left shoulder and then gradually involved the chest, face and the upper limbs.

On examination on September 22, he was afebrile; there were erythematous non-tender, circinate plaques 4–5 cm in diameter on the back of the chest, face, shoulders and forearms. The lesions were not anaesthetic and there was no evidence of leprosy.

Investigations showed ESR of 144 mm/h; WBC of 7,000/mm^3 (74% neutrophils, 2[illegible]% lymphocytes, and 4% eosinophils); Hb 9·8 gm/100 ml, P.C.V. 34·1%, M.C.H.C. 29·1%. The blood film showed normocytic hypochromic red cells and toxic granulation of white cells. L.E. cell test was negative.

Urine showed albumin and red cells on 21 September but these had disappeared by 23 September. Chest X-ray was clear.

A skin biopsy on 25 September showed focal areas of spongiosis; there were areas of focal perivascular infiltration of the upper and mid dermis, consisting mainly of polymorphs with some lymphocytes and histiocytes.

The patient was observed for 2 days. He developed a slight evening pyrexia, and the skin lesions became more dusky and pruritic. On 27 September, he was given prednisolone 10 mg thrice daily and was discharged on 10 mg twice daily on 30 September. By 7 October all skin lesions had cleared. Prednisolone was tailed down and discontinued on 13 November. He had no recurrences and was well when seen on 21 February 1973.

Summary of the clinical findings in the eighteen patients

Incidence. The disease occurred in nearly four per thousand new cases seen at the Dermatological Unit. There appears to be a seasonal bias, in that nearly half the cases were seen during the months of January and February. These months follow the heavy monsoonal rains and are the coldest (mean temperature 23·4°C) and the driest (mean rainfall 2·77 in per month, mean relative humidity 64%) months of the year.

Sex and age distribution. All but one patient were female. Their ages ranged from 30–75 years. The mean age was 49 years.

Preceding illness. All patients had a history of an illness or an injury 2–7 days prior to the appearance of the characteristic eruption. Twelve had symptoms suggestive of an upper respiratory tract infection; three had bronchitis in addition. *Streptococcus haemolyticus* was isolated from three of the four patients who had inflamed throats at the time of examination. Three patients had an influenza like preceding illness. In two patients the skin eruption followed cellulitis complicating vaccination. The skin lesions appeared following an infected abrasion in one patient.

In fifteen of the patients the preceding illness was severe enough to require medication with analgesics, antihistamines, antimicrobials and bronchodilators. There was no evidence to suggest a causal relationship between the drugs and the dermatosis; nor did these drugs have any effect on the course of the illness. No patient had received steroids prior to observation by us.

Fever. The patients came under our care between the 3rd and 42nd day of their illness. Fifteen patients had fever at some time during the course of the disease, nine at the time of examination. The highest temperature recorded was 39·5°C. Five had temperatures of over 37·8°C. The temperature was remittent and higher in the evening.

Skin eruption. All patients had lesions with the characteristic morphology as described by Sweet (1964) and by Crow *et al.* (1969) (Fig. 2).

In nine patients the lesions were tender, the palmar and plantar lesions being exquisitely so. The size ranged from 0·5 to 7·0 cm in diameter. The number of lesions varied from 7 to 15. The lesions were found on the arms and dorsa of the hands in fifteen patients, upper chest in ten, legs and dorsa of the feet in nine, neck and shoulders in eight, palms and/or soles in five, face and ears in four. No lesions were found between the lower chest and the thighs. The lesions were bilateral though asymmetrical, appearing between 2 and 7 days (mean 4 days) from the onset of the illness.

Eye signs. Thirteen patients had 'red eyes' at the time of examination. Signs were unilateral in three,

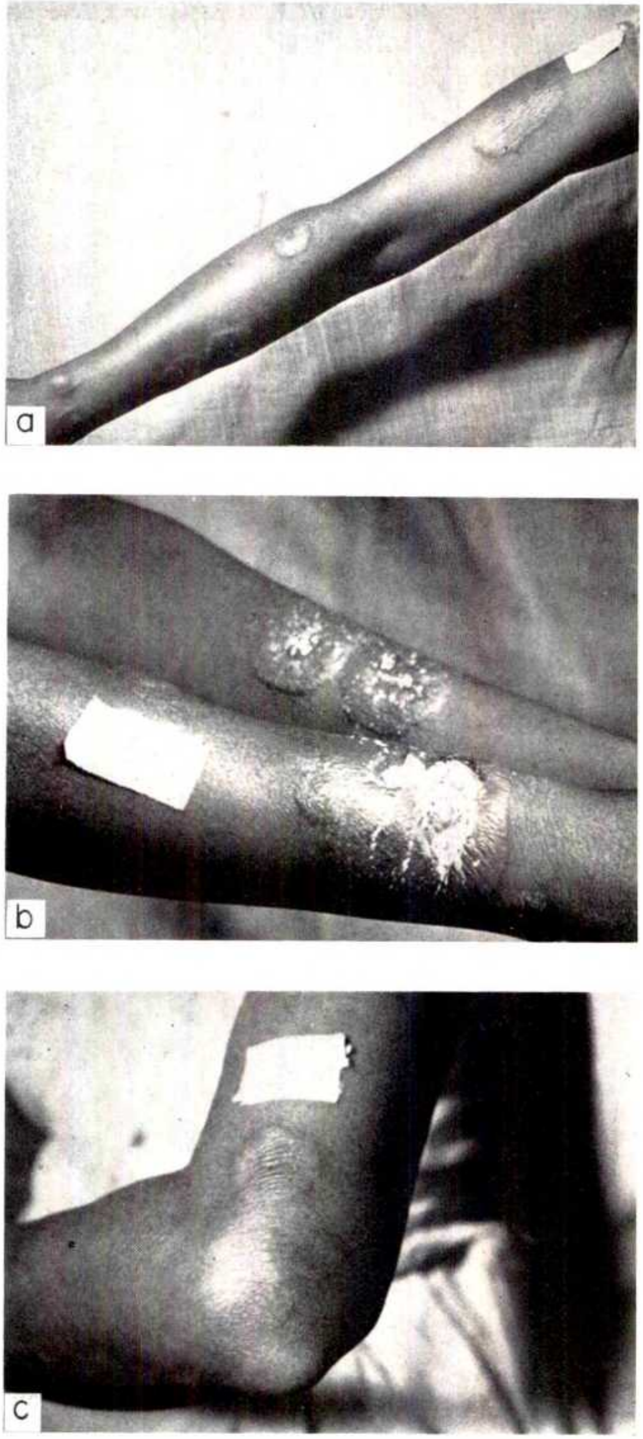

FIGURE 2. The characteristic lesions of Sweet's syndrome—large plaques with partial central clearing. In (b) the central clearing is seen through the calamine lotion applied by the patient.

bilateral in the others with a tendency to asymmetry. Eight had conjunctivitis and five had episcleritis. The eye signs appeared within the first 10 days of the illness and usually followed the skin lesions by 1–6 days. In one patient they preceded the appearance of the eruption by 1 day.

Joint involvement. Nine patients developed joint symptoms during the illness. In one patient (case 5) these developed later. All these patients complained of pain and stiffness of the joints, mainly of the cervical spine, hip, knee, ankle, shoulder and elbow. Again there was a tendency to asymmetry. The cervical spine was involved in the older patients (aged 60, 70, and 75 years). Joint effusion was found in one case who had a history of recurrent joint pains for the previous 15 years. Joint symptoms usually developed 2–10 days from the onset of the illness.

Leukocytosis. Total white cell count ranged from 4,800/mm^3 to 24,000/mm^3 and the absolute neutrophil count ranged from 3,352/mm^3 to 19,920/mm^3. Ten patients had an absolute neutrophil count of over 7,500/mm^3. Those patients who had fever and tenderness of the lesions usually showed a moderate to high neutrophilia.

Erythrocyte sedimentation rate. All but one patient had an ESR (Westergren) of over 35 mm/h. The report for one case was not available. The highest was 144 mm and the lowest was 10 mm. Ten patients had an ESR of 90 mm and over. It is interesting to note that all patients who had a leukocytosis, eye involvement and joint symptoms simultaneously had an ESR of over 90 mm. The patients with high fever and tender lesions often had a high ESR.

Response to corticosteroids. Patients showed no response to penicillin, streptomycin, or tetracyline given in full dosage. Nine patients who had marked constitutional symptoms were given prednisolone, in an initial dosage of 30 mg daily and tailed off over a period of 1–6 weeks. Five of these patients received the drug for 2 weeks. Constitutional symptoms, fever, pain and tenderness of the lesions improved dramatically within 48 h. Eye signs and joint symptoms disappeared and the skin lesions flattened in 3–12 days.

Duration and relapses. The total duration of the illness varied from 3 to 9 weeks. In fourteen patients the lesions cleared in 3–4 weeks. The average duration of the disease (i.e. from the onset of the first symptom to the disappearance of the skin signs) was 3·8 weeks for those who were treated with steroids and 4·9 weeks for those from whom steroids were withheld. None of the patients had any relapses nor any scarring during the period under observation.

Other findings. Two cases showed a transient albuminuria and microscopic haematuria. Two cases had hypergammaglobulinaemia. Twelve patients had a haemoglobin of less than 12·8 g/100 ml. Four had a normocytic normochromic anaemia, four had a microcytic hypochromic anaemia, three had a normocytic hypochromic anaemia and one had a macrocytic hypochromic anaemia. None had a positive latex fixation test, L.E. cells in the blood or evidence of chronic lung disease on chest X-ray.

Histology. A detailed presentation of the histological findings in the eighteen patients is outside the scope of this paper. However, the histological changes were fairly uniform, the details varying with the age of the lesion at the time of biopsy. This dynamic nature of the histological changes is illustrated in case 1. The general observations were that the epidermis was not markedly involved, the only changes being mild spongiosis in the early stages followed by acanthosis and mild hyperkeratosis. The dermal oedema and cellular infiltration were maximal in the upper and mid dermis. The infiltrate was focal, being most intense around blood vessels. The cell type varied; neutrophils predominated in the early stages, whereas histiocytes and mononuclears were found in the late stages. The polymorphonuclear infiltrate was associated with marked leukocytoclasis. Fibrinoid changes, leukocyte-platelet thrombi in the vessels, tissue necrosis and hyaline changes in the collagen were not observed.

DISCUSSION

A total of forty-three cases considered to be Sweet's syndrome have been documented so far; this includes the eighteen cases presently reported. The clinical presentation of these cases varied from an acute and severe systemic illness (Frayha *et al.*, 1972) to a chronic, recurrent and isolated cutaneous lesion (Sweet, 1968; Whittle *et al.*, 1968). The patients in our series occupied the middle range of this spectrum. Even within this range there were striking variations in the clinical presentations. Five cases were severely ill while two cases had minimal constitutional symptoms, so that in-patient treatment was not sought.

As the clinical spectrum of Sweet's syndrome is wide, the condition may be confused with a large number of other diseases. The provisional diagnosis made by the physicians at the time of referral to us included lymphangitis, cellulitis, erysipelas, erysipeloid, erythema nodosum, leprosy, lepra reaction, allergic vasculitis and 'allergic reaction' to aspirin, penicillin or smallpox vaccination. In addition septicaemia, dermatomyositis, systemic lupus erythematosus, erythema multiforme, erythema elevatum diutinum, granuloma faciale, relapsing nodular panniculitis and other variations of vasculitis could be included in the differential diagnosis.

Since the four cardinal features laid down by Sweet (1964) were not invariably found in all our patients, the following criteria were used to differentiate Sweet's syndrome from the conditions mentioned above.

(1) A cutaneous eruption with the characteristic morphology as described by Crow *et al.* (1969).

(2) Failure of antibiotics to alter the course of the disease, but a rapid and dramatic response to systemic corticosteroids.

(3) Complete resolution of the lesions without scarring.

(4) Histologically: dermal oedema; an inflammatory infiltrate, greatest around the vessels of the upper and the mid dermis, consisting of a variable proportion of mature polymorphs, lymphocytes and other inflammatory cells; and an absence of discernible damage to the vessel walls or the connective tissue.

The frequency of other important clinical features of this disease is shown in Table 1. It is clear that fever and leukocytosis are not the commonest features observed, either in our series or in the previously reported cases. However, the four criteria mentioned above were found in the previous cases whenever this information was documented. Because of the consistency of these features, like Sweet and Crow *et al.*, we believe that this illness is a distinctive entity. Moreover, apart from the 'intellectual satisfaction of being able to classify the disease' (Sweet, 1964) it is important to recognize this clinical entity, because the diagnosis of Sweet's syndrome means the recognition of an essentially benign condition, which has a tendency to natural resolution, and this resolution may be hastened if necessary by corticosteroids. As this dermatosis is neither always acute, nor always febrile, we feel that the eponym 'Sweet's syndrome' is preferable to the rather cumbersome term 'acute febrile neutrophilic dermatosis'.

Although the aetiology and the pathogenesis of Sweet's syndrome are obscure, the analysis of the clinical findings of the recorded cases indicates some directions for future investigations. The localization of the lesions to the exposed parts of the body, to sites of previous trauma (Sweet, 1968) and to scars (Crow *et al.*, 1969) suggests that connective tissue previously damaged in some way (e.g. sunlight, trauma) may be necessary for such localization. Further the greater predilection of the disease to

TABLE 1. Percentage occurrence of clinical features in Sweet's syndrome*

	Previous series	Present series
Skin eruption	100% (25/25)	100% (18/18)
Response to steroids	100% (19/19)	100% (10/10)
Preceding illness	95% (20/21)	100% (18/18)
Raised ESR	87% (13/15)	94% (16/17)
Fever	83% (20/24)	83% (15/18)
Eye involvement	4% (1/25)	72% (13/18)
Leukocytosis	79% (19/24)	59% (10/17)
Joint involvement	12% (3/25)	56% (10/18)
Albuminuria	16% (4/25)	11% (2/18)

* Cases where the information regarding the clinical features was not clear from the literature or was not available are not included.

females and the 10 year difference in mean ages between the males and females (57 years and 47 years respectively for the forty-three reported cases) may be due to earlier degeneration of dermal collagen in the female (Shuster & Bottoms, 1963). The prompt response of the disease to corticosteroids and the association of joint, eye and kidney involvement, as seen in serum sickness like disorders, points to an underlying immunological disturbance. The intense neutrophilic reaction seen in the lesions may indicate that this immunological disturbance is a Type III allergy (soluble complexes).

Since the appearance of the skin lesion follows closely on an upper respiratory tract or skin infection, an infective agent may be linked with Sweet's syndrome. If this were a single agent, the haemolytic streptococcus appears to be the most likely organism. However, conclusive evidence of a preceding streptococcal infection, in the form of an elevated ASOT, C-reactive protein and a positive throat swab culture was found in only three of the previously reported cases and in three of our patients.

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Cell migration and DNA synthesis in organ culture of human skin

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SUMMARY

Cell migration and DNA synthesis in organ cultured skin were studied. Cell migration leads to the formation of a new epithelial layer around the free edges of the dermis. No significant DNA synthesis occurs in this newly formed epithelium. In contrast, DNA synthesis in the original epidermis is maintained throughout the period of culture as shown by the uptake of radioactive thymidine.

Addition of hydrocortisone to the culture medium increases cell migration, and phytohaemagglutinin, while dramatically stimulating mitotic activity, suppresses cell migration.

These results show that an increase in epithelial cell division is accompanied by diminished cell migration whereas an increase in cell migration occurs in the presence of a decrease in epithelial cell division. The behaviour of skin in organ culture during the first 48–72 h can be compared with that seen in wound healing.

Epidermal cell migration is one of the characteristic features of skin successfully maintained in organ culture. The migrating cells can be seen to cover the free edges of the dermis of the explant and frequently complete encapsulation by epidermal cells (epiboly) takes place within 24–48 h. Parakeratotic cells may also appear in the newly formed layers (Sarkany, Grice & Caron, 1965).

No studies on cell division and DNA synthesis in the newly formed layers (epiboly) have been published. However, the origin of the migrating cells and their increase in numbers have been studied in wound repair experiments where the migration and division of cells have been considered to be comparable to the changes seen in organ culture (Gibbins, 1969). While studying the fate of radioactively labelled thymidine in organ culture, we have made some detailed observations on the behaviour of the migrating cells *in vitro* and we have also investigated the effects of hydrocortisone and phytohaemagglutinin on cell migration.

MATERIALS AND METHODS

Adult human skin was obtained from patients undergoing surgical removal of tattoos, using Castroviejo's keratotome (Blank, Rosenberg & Sarkany, 1961). Explants of adjacent normal skin 0·5 mm thick were maintained on human serum and were cultured in the presence of neomycin (100 μg/ml) on waxed lens tissue rafts with air as the gas phase, as previously described (Sarkany *et al.*, 1965). These were incubated in the presence of hydrocortisone hemisuccinate (60 and 100 μg/ml) and phytohaemagglutinin M (Burroughs Wellcome). The phytohaemagglutinin was made up to 5 ml with normal saline and 0·1 ml was added to each 2 ml of serum. Appropriate normal controls were cultured together with the above experimental explants for up to 14 days. A total of 200 explants obtained from eleven individuals were used. ^{3}H thymidine (1 μCi/ml, with an activity of 2·0 Ci/mM) was added for the last 4 h of culture in sixty explants. Sections 4 μm thick were cut and autoradiography was carried out using Kodak AR 10 stripping film. The films were developed after 3 or 6 weeks exposure and the slides were stained with haematoxylin.

In a second group of experiments involving thirty-eight explants, cultures were labelled sequentially, using ^{3}H and ^{14}C thymidine. There is no absolute need to use a label at two different times during the period of culture, since the same information could be obtained from two separate labelling experiments. However, labelling with ^{3}H and ^{14}C thymidine at different times in the same culture clearly demonstrated differences in uptake at different times in the same explant. Because of the high activity of the radioactive material, only small volumes of media were used and the culture methods were therefore altered. Skin slices were incubated for 7 h with ^{14}C thymidine (0·01 mCi/ml, with an activity of 60 mCi/mM) in the presence of neomycin (50 μg/ml), floated on the surface of the medium, using surface tension forces to support them, and 5% CO_2 in air was used as the gas phase, as previously described (Sarkany & Gaylarde, 1970). The explants were washed and maintained on label-free medium for 16 or 64 h, then ^{3}H thymidine (0·05 mCi/ml, with an activity of 5·0 Ci/mM) was added for the last 3 h of culture. The labelled cultures were placed in 5% trichloracetic acid at 4°C for 2 h. The explants were washed in saline, dehydrated, and embedded in wax for histology. Sections 4 μm thick were cut and two layers of Kodak AR 10 stripping film were applied. The film was left for 10 days, developed and stained with haematoxylin.

Since the maximum range of penetration of the β radiation of tritium into a gelatine film is less than 3 μm, whereas the maximum penetration of the radiation from ^{14}C is 300 μm, the emissions can be differentiated. The thickness of Kodak AR 10 film is greater than 3 μm and therefore the use of two layers enables one to distinguish the two emissions, since ^{14}C labelled cells will expose both layers of film whereas ^{3}H thymidine will affect only a single film layer.

RESULTS

The double labelling of explants with ^{3}H and ^{14}C thymidine demonstrated the difference between DNA synthesis in the original epidermis and that occurring in the newly formed cell layer (Figs. 1, 2 and 3). There was no uptake of ^{3}H thymidine by the migrating cells, demonstrating that there was no DNA synthesis occurring in this layer. A high proportion of these cells were labelled with ^{14}C during the first 7 h of the culture which showed that DNA synthesis must have taken place before migration started. Cells labelled with ^{14}C were identified by the diffuse and scattered appearance of the silver grains, which were present in both film layers. This made exact counting of labelled nuclei liable to some error where the distance between nuclei was small. On the other hand, ^{3}H labelled nuclei were easily distinguishable because of good localization of silver grains in the film in immediate apposition to the skin section.

The incorporation of ^{3}H thymidine at the end of the culture period in explants cultured for up to 14 days was almost exclusively limited to the epidermal cells of the original epidermis. Only a few sparsely labelled nuclei were seen in the newly formed epithelium and the labelling index was greatest in the initially present epidermis within 1 mm of the cut edge.

The presence of hydrocortisone in the culture medium caused a statistically significant increase

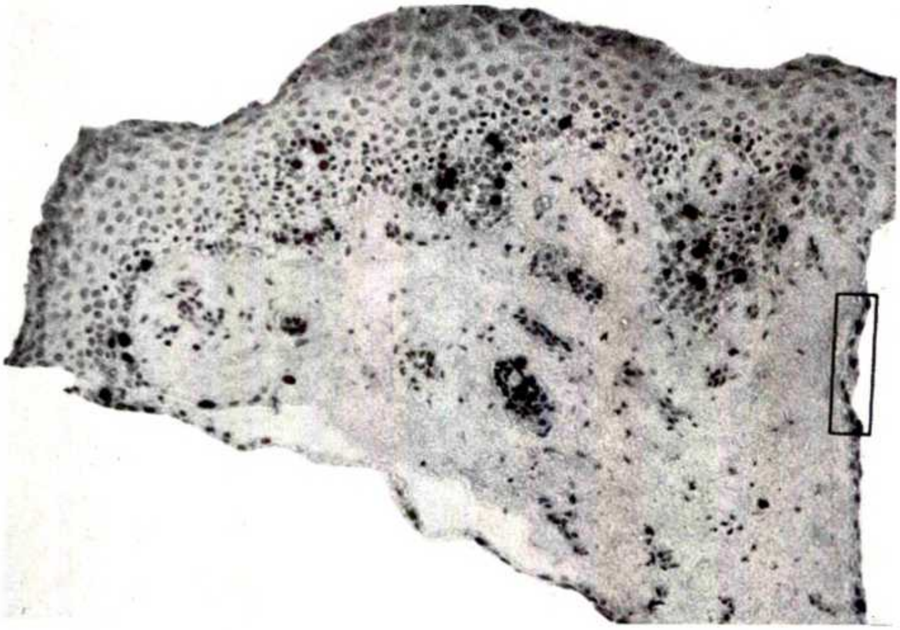

FIGURE 1. Explant showing complete epiboly after 26 h in culture. Note densely labelled cells in original basal and suprabasal regions labelled with ^3H thymidine over last 3 h of culture. More diffusely ^{14}C labelled cells can only be seen clearly at higher magnification (× 140). The section shown is from the edge of an explant, since the size of these explants was 3–4 mm square and at the magnification of this illustration the whole section could not be shown.

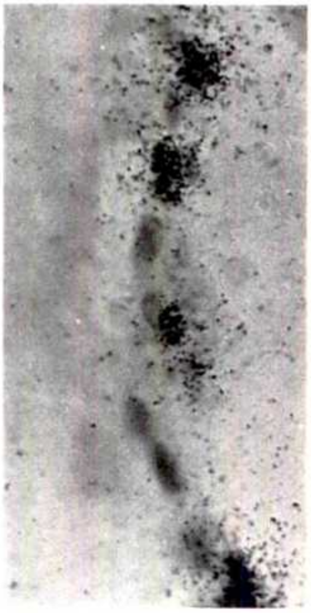

FIGURE 2. Inset from Fig. 1 showing ^{14}C labelled nuclei. Note scattered distribution of silver grains around the nuclei. This contrasts with the circumscribed nature of distribution of the silver granules over ^3H labelled nuclei shown in Fig. 3 (× 600).

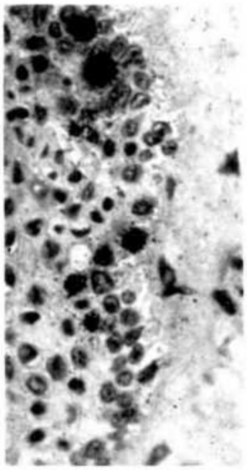

FIGURE 3. ^{3}H labelled nuclei in original epidermis showing discrete localization (× 300).

TABLE 1. Epiboly in explants cultured in the presence of hydrocortisone and phytohaemagglutinin

	Hydrocortisone (60 and 100 μg/ml)		Hydrocortisone control		Phytohaemagglutinin control		Phytohaemagglutinin-M (1/20 dilution of vial)	
Cell migration	Number of explants	% of total number of explants	No. of explants	% of total number of explants	No. of explants	% of total number of explants	No. of explants	% of total number of explants
None	6	15·0	15	53·6	22	36·7	29	40·2
Partial cover of free dermis by new epithelial cells	23	57·5	10	37·7	30	50·0	40	55·6
Complete encapsulation of dermis by new epithelium	11	27·5	3	10·7	8	13·3	3	4·2

($P<0{\cdot}01$) in cell migration (Table 1) and preserved the normal appearance of the stratum spinosum as compared with control cultures ($P<0{\cdot}05$). Other features, statistically not significant ($P<0{\cdot}22$ $>0{\cdot}05$), were also noted and included a decrease in the incorporation of thymidine, reduction of mitoses in the epidermis, improved preservation of stratum granulosum and suppression of the development of parakeratosis which normally occurs in explants cultured *in vitro*. Phytohaemagglutinin diminished cell migration ($P = 0{\cdot}07$) (Table 1). The statistical analysis of the results, which are summarized in the Table 1 was made using the paired t test. It should be noted that it is possible to show much higher levels of significance than those reported above by simple comparison of the proportion of cultures showing complete epiboly between the test and control cultures (Table 1). Because of the variation of the period of culture, and the origin of the explants from eleven different human subjects, it was only considered valid to use the paired t test. This meant comparing only results of explants obtained from the same individuals and maintained for the same period of time.

DISCUSSION

These studies of cell migration in explants of adult human skin have shown that there is no significant DNA synthesis in cells of the newly formed epithelial layer in culture and that thymidine uptake in the original epidermis is maintained for up to 14 days. The addition of hydrocortisone to the culture medium improves cell preservation and favours an increase in cell migration. On the other hand, phytohaemagglutinin appears to inhibit the migration of epithelial cells *in vitro*.

The behaviour of skin in organ culture has been considered to reflect changes seen in experimentally produced wounds. The migration of cells takes place at similar rates both *in vivo* and *in vitro* (Gibbins, 1969) and the mitotic response to wounding parallels the changes seen *in vitro*. Gelfant (1960) demonstrated that there is a large increase in mitotic activity within 1 mm of the cut edge of skin maintained for 5 h *in vitro*. We have observed a similar distribution of both mitotic figures and thymidine uptake in skin maintained in culture for up to 14 days.

The lack of DNA synthesis and cell division in the migrating layers also shows close resemblance to the situation occurring *in vivo* in wounds. Little or no mitotic activity is seen in the newly formed epithelial layer of experimental wounds until 48–72 h after migration begins (Ordman & Gillman, 1966; Gibson, 1964; Chiakulas, 1952). The outgrowth of epithelial cells from skin cultures was considered to be almost entirely migratory in other studies (Hsu, 1952; Matoltsy & Sinesi, 1957) and mitotic activity was absent in the outgrowth. The high proportion of cells of the migrating layer labelled with ^{14}C thymidine found in this study is of note. This presumably supports a conclusion that there had been proliferation of the cells at least through the phase of DNA synthesis prior to migration. The cell cycle in the human epidermis has been estimated to last about 300 h, of which in normal skin the phase prior to DNA synthesis (G1) forms the greatest part, i.e. some 284 h (Weinstein & Frost, 1968). The next phase of DNA synthesis (S) lasts 16 h. The high proportion of cells in the migrating epithelium labelled in our experiments suggests that a large proportion of these cells had been made to enter the S phase prior to migration, perhaps as a result of stimulation by the special conditions of the culture.

Phytohaemagglutinin causes a dramatic increase in cell division (Sarkany & Caron, 1965) and in contrast to hydrocortisone, the normal cellular features are lost and cells appear flattened. The thickness of the basal and suprabasal layers is reduced. The present results suggest that phytohaemagglutinin may partially inhibit cell migration ($P = 0{\cdot}07$). Since the migrating cells do not synthesize DNA or divide, it is possible that these two agents affect migration by increasing or decreasing the population of non-dividing cells. It has been shown (Caron, 1969) that hydrocortisone hemisuccinate in organ culture inhibites cell migration. The concentrations of hydrocortisone hemisuccinate were greater than those used in our experiments and varied between 200 and 2000 μg/ml. Concentrations over 1000 μg/ml caused necrosis, and at 2000 μg/ml cell migration was never observed. At the lowest concentration, no difference from control cultures was noted. The effect of hydrocortisone on wound healing *in vivo* is to inhibit collagen formation. However, in one study the effect of hydrocortisone on epithelial migration has also been investigated (Boulas, 1959). Experimentally induced wounds were irrigated with a suspension containing 25 mg/ml of hydrocortisone and the rate of epithelialization and formation of granulation tissue was observed for up to 154 days. The presence of hydrocortisone delayed the complete epithelialization of the wounds but the epithelial cell migration (seen microscopically) 7 days after wounding was similar to that in control wounds. The difference between these and our findings shows that very high concentrations cause epithelial cell necrosis and suggests that the inhibition observed at moderately high concentrations may be due to a toxic effect.

The effect of metabolic inhibitors on cell migration in organ culture has been studied (Gibbins, 1972). Agents which inhibit glycolysis also inhibit cell migration. Inhibitors of the Kreb's cycle had no effect and compounds blocking electron transfer in the cytochrome chain, e.g. cyanide, increased cell migration. Acceleration of repair of areas denuded of epidermis would be useful in many clinical situations. The increased cell migration observed *in vitro* in response to hydrocortisone and sodium cyanide has no practical application. Hydrocortisone inhibits healing in clinical use and the effect of sodium cyanide is even more unlikely to be of use.

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Prostaglandins and cyclic AMP in epidermis

EVIDENCE FOR THE INDEPENDENT ACTION OF PROSTAGLANDINS AND ADRENALINE ON THE ADENYL CYCLASE SYSTEM OF PIG AND HUMAN EPIDERMIS, NORMAL AND PSORIATIC

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SUMMARY

Prostaglandins E_1 and E_2 stimulate cyclic AMP accumulation in pig epidermis and in human epidermis from patients with psoriasis. Prostaglandins A_1, A_2 and $F_{2\alpha}$ are relatively ineffective. The fact that this stimulation is not inhibited by a β-blocker (propranolol) and that the stimulation by prostaglandin E_2 and adrenaline is additive indicates that each drug acts independently on the epidermal adenyl cyclase system. In other words, prostaglandins E_1 and E_2 act on a site other than the β-receptor of adenyl cyclase in epidermis. The stimulation by prostaglandins E_1 and E_2 is not additive; hence they probably act on the same site. Concentrations of prostaglandin E above 3×10^{-7} M are effective in causing stimulation. This concentration may be within the physiological range and the contribution of endogenous prostaglandin levels in the control of intracellular cyclic AMP levels cannot be disregarded.

The adenyl cyclase system in mammalian cells is activated by a variety of hormones and prostaglandins. This system, which is localized in the plasma membrane, plays an important biological role as a regulator of cell metabolism and perhaps cell division. Although the adenyl cyclase system has not been purified, it is now generally accepted that it is composed of regulatory and catalytic subunits; the former for the specific recognition of a hormone (a receptor) and the latter for the catalytic conversion of adenosine 5′-triphosphate to cyclic AMP. One hormone, adrenaline, has been shown to activate epidermal adenyl cyclase (Brønstad, Elgjo & Øye, 1971; Powell, Duell & Voorhees, 1971; Duell *et al.*, 1971; Marks & Rebien, 1972; Hsia *et al.*, 1972).

Not withstanding the early discovery of prostaglandins (Goldblatt, 1933; Von Euler, 1935), their relation to the cyclic AMP system has only recently been suggested; for example, Steinberg *et al.*

(1964) demonstrated the antagonistic role of prostaglandins toward the lipolysis caused by adrenaline, adrenocorticotropic hormone, or thyroid-stimulating hormone. As far as skin is concerned, only very limited and recent studies indicate that prostaglandin E (PGE) increases the cyclic AMP level in epidermis (Hsia, Wright & Halprin, 1973; Aso *et al.*, 1974); however, its mode of action has not been studied in detail. The purpose of this report is to present basic data on how prostaglandins affect the adenyl cyclase system in the epidermis.

MATERIALS AND METHODS

Epidermal slices were taken by a keratome from pigs and human volunteers. This procedure has been described in detail previously (Yoshikawa *et al.*, 1975a, b). No anaesthetics were used. The resulting sheet of epidermis was cut into 5 × 5 mm squares and preincubated in Hank's medium for 15 min at 37°C in order to standardize the initial cyclic AMP level in the epidermal pieces. This procedure is necessary because the level of cyclic AMP increases immediately after the removal of epidermis from the subjects (Yoshikawa *et al.*, 1975b) and at the same time that of the precursor (adenosine 5′-triphosphate) decreases after the removal of epidermis. The levels return to normal after about 15–20 min. Two of the epidermal squares were randomly selected and carefully floated with their keratin layers up on Hank's medium containing different concentrations and varieties of prostaglandins and 2 mM theophylline. After a 5-min incubation at 37°C, they were quickly frozen between plates of dry ice. The cyclic AMP content in these skin slices was measured by Gilman's protein binding method (1970) with minor modifications in the method of extracting cyclic AMP (Yohiskawa *et al.*, 1975a, b). The binding assay was done in duplicate. Protein was measured by the method of Lowry *et al.* (1951). The prostaglandins* were prepared fresh before each experiment and were dissolved in ethanol. The final concentration of ethanol in the media was 0.1%; the control group (no prostaglandin additions) also contained 0·1% ethanol, which at this low concentration normally does not affect the cyclic AMP level in epidermis. Theophylline (2 mM) by itself does not increase the cyclic AMP level of floating skin slices. However, when adenyl cyclase is stimulated either by adrenaline or prostaglandin, the concentration of cyclic AMP within the cells reaches a higher and more sustained peak in the presence of this phosphodiesterase inhibitor.

RESULTS AND DISCUSSION

Effects of prostaglandins

All basic experiments were performed with pig epidermis. The effects of various prostaglandins are shown in Fig. 1. Stimulation by PGE_1 and PGE_2 was marked and about equal. Prostaglandins A_1, A_2 and $F_{2\alpha}$ stimulated adenyl cyclase only slightly. The time course of PGE_2 stimulation is shown in Fig. 2. Cyclic AMP accumulated rapidly for 4–5 min and then decreased but did not return to the baseline value by 15 min. This decrease was probably due to the activation of cyclic AMP-phosphodiesterase by the elevated cyclic AMP (Schultz & Daly, 1973), which was not completely inhibited by the theophylline added to the medium at a final concentration of 2 mM. The short incubation periods (5 min) used in our experiments give sufficient time for adenyl cyclase activation and consequent cyclic AMP accumulation, and avoid the uncertainties in later time periods due to the unpredictable activation of the phosphodiesterase. The effects of various concentrations of PGE_2 are shown in Fig. 3. The activation is dose dependent and saturates at a concentration of approximately 3×10^{-6} M.

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* The generous gift of Dr J.E.Pike, The Upjohn Co., Kalamazoo, Michigan.

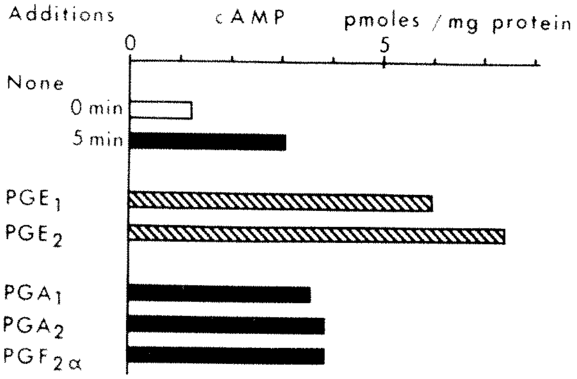

FIGURE 1. Effects of prostaglandins on cyclic AMP in pig epidermis. Final concentration of prostaglandins was 3 × 10^{-6}M each. Epidermis was incubated for 5 min at 37°C. For detailed experimental conditions, see text.

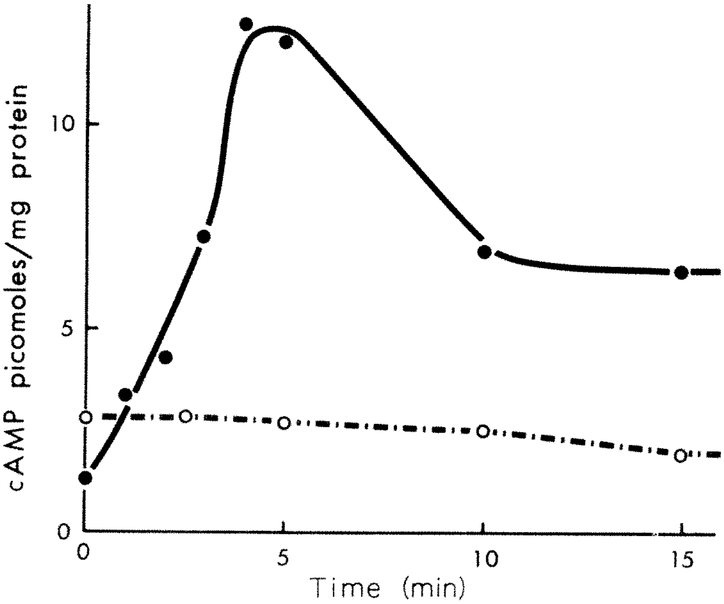

FIGURE 2. Time course of PGE_2 stimulation on epidermal cyclic AMP accumulation (pig). PGE_2 concentration was 3 × 10^{-6}M with final ethanol concentration of 0·1%. A dotted line (- - - o - - -) shows a control experiment done separately without the addition of PGE_2 but with 0·1% final concentration of ethanol.

Prostaglandin and adrenaline stimulatory sites

Since both PGE and adrenaline cause a rapid increase in cyclic AMP in epidermis, their mode of action and possible interrelation were studied. The various possibilities are considered as follows: (1) adrenaline may stimulate PGE synthesis, which then activates adenyl cyclase to cause cyclic AMP accumulation; (2) PGE may stimulate adrenaline release which then activates adenyl cyclase to cause cyclic AMP accumulation; or (3) both PGE and adrenaline act independently on adenyl cyclase. This last possibility also includes a further one (3a), namely, that adrenaline binds to a

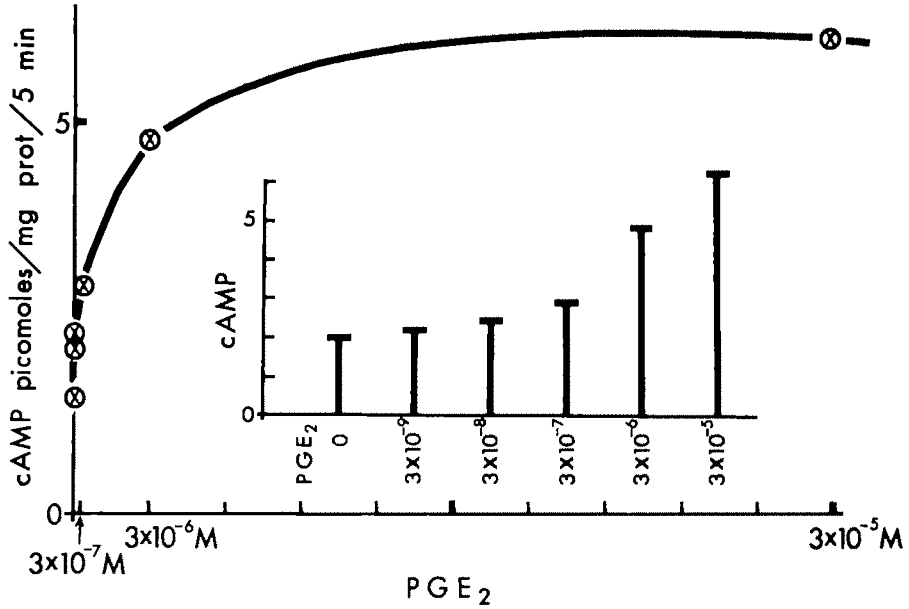

FIGURE 3. Effects on cyclic AMP formation in pig epidermis varying PGE$_2$ concentration in the incubation medium. PGE$_2$ concentrations are shown in abscissa from 3×19^{-9}M to 3×10^{-5}M.

receptor site of adenyl cyclase and that PGE acts directly on the catalytic site of adenyl cyclase. (However, we did not intend to resolve (3) from (3a) in this report).

Should mechanism (1) be correct, the administration of an inhibitor of PGE synthesis, such as indomethacin or salicylate, should block the cyclic AMP accumulation in the epidermis caused by adrenaline. The results shown in Fig. 4 clearly refute this, since neither inhibitor affects the stimulation of adenyl cyclase by adrenaline. In addition, it should be noted that indomethacin or salicylate alone does not stimulate cyclic AMP formation.

Should pathway (2) be correct, the addition of a β-blocker such as propranolol should stop the increase in cyclic AMP caused by PGE. The results in Fig. 5, however, show that the addition of propranolol did not significantly decrease the cyclic AMP level that had been increased by PGE$_2$. This fact suggests that the β-receptor is not the site of PGE action. It is in remarkable contrast to the effect of the β-blocker on adrenaline stimulation (Fig. 6), where the addition of the β-blocker decreases the cyclic AMP level to about the 5-min level of the control. By simple deduction, therefore, either (3) or (3a) must be regarded as the likely process in the epidermis. Evidence that the two sites are different can also be found in Fig. 7. The sum of the increase by PGE$_2$ and that by adrenaline has to be equal to the cyclic AMP level that is increased by the simultaneous addition of PGE$_2$ and adrenaline, if the two sites are different and therefore are additive. Fig. 7 demonstrates that the effects of PGE$_2$ and adrenaline are either additive or slightly synergistic; the results suggest independent sites (and with a possibility of an additional weak allosteric effect) for PGE$_2$ and adrenaline. Fig. 8 further demonstrates that adding a β-blocker to the [PGE$_2$+adrenaline] eliminated the adrenaline effect and resulted in the PGE$_2$ effect alone. The addition of an α-blocker, such as priscoline at 10^{-4}M final concentration, showed no effect on PGE$_2$ activation.

Prostaglandins and psoriatic epidermis

Generally the response of human epidermis to prostaglandins appears to be similar in nature and

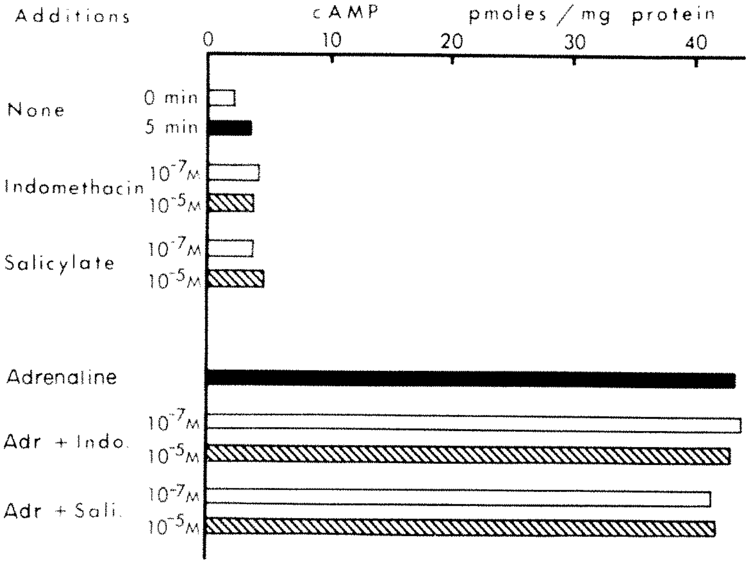

FIGURE 4. Effects of inhibitors of PGE biosynthesis on cyclic AMP formation in pig epidermis. Cyclic AMP levels shown are those after a 5-min incubation at 37°C. Adrenaline concentration, when added, was 5×10^{-6}M. Cases I–IV represent data from different pigs.

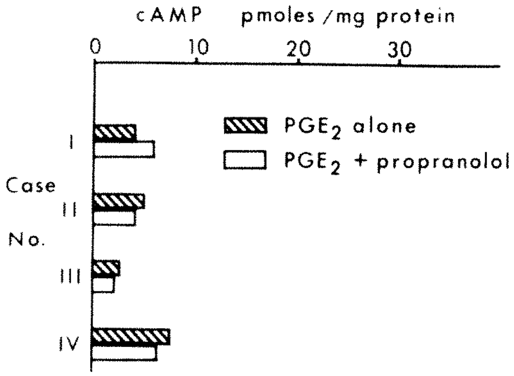

FIGURE 5. Effects of PGE_2 and (PGE_2 + β-blocker) on cyclic AMP formation. Cyclic AMP levels shown in this figure are those stimulated by PGE_2 during a 5-min incubation period at 37°C. The control values (the levels at 5 min without PGE_2) have been subtracted from total cyclic AMP level at 5 min in the presence of PGE_2. Final concentrations of PGE_2 were $3{\cdot}3 \times 10^{-5}$M in Case I and 3×10^{-6}M in other experiments; those of propranolol 10^{-3}M in Case I and 10^{-4}M in others.

extent to that of pig epidermis. Neither psoriatic nor uninvolved epidermis from the same patients responded significantly to prostaglandins A_1 and $F_{2\alpha}$ (Fig. 9). On the other hand, adenyl cyclase was stimulated to various degrees by PGE_1 and PGE_2; i.e. after a 5-min incubation, the cyclic AMP level was increased two to fourfold in the psoriatic and five to ninefold in the uninvolved epidermis. The simultaneous addition of PGE_1 and PGE_2 does not yield an additive effect on cyclic AMP accumulation and the data suggest the same action site for both compounds. Propranolol, a β-blocker, does not inhibit PGE_2 stimulation in either psoriatic or uninvolved epidermis (Fig. 9, Case I), as it does not in pig epidermis. When propranolol is added to [PGE_2 + adrenaline], the level

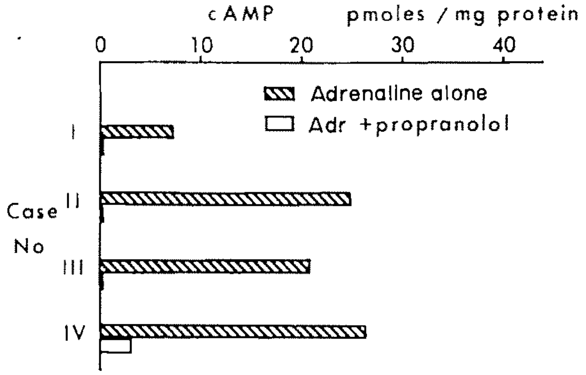

FIGURE 6. Effects of adrenaline (Adr) and [adrenaline+β-blocker] on cyclic AMP formation. The experimental conditions were the same as for Fig. 5 except for the concentrations of adrenaline ($2{\cdot}3\times10^{-5}$ in Case I and $4{\cdot}5\times10^{-6}$M in others) used instead of PGE_2.

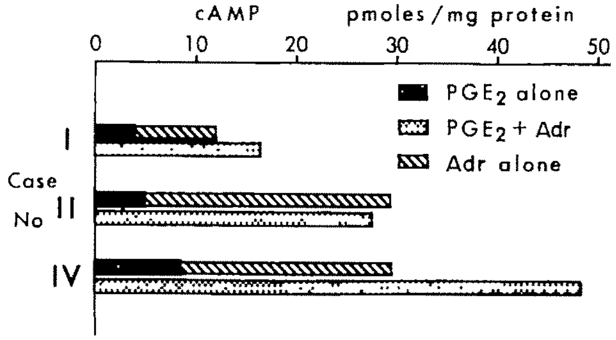

FIGURE 7. Effects of simultaneous addition of PGE_2 and adrenaline (Adr) on cyclic AMP formation. Basic experimental conditions were the same as for Figs. 5 and 6. The stimulation of cyclic AMP by the simultaneous addition of two compounds was compared with the sum of the stimulation caused by each drug alone.

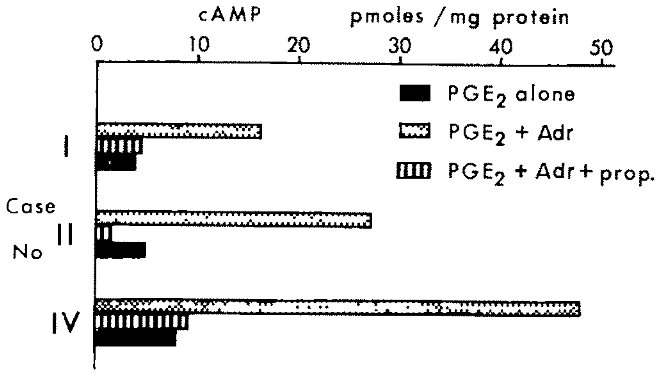

FIGURE 8. Effects of a β-blocker on the stimulation of cyclic AMP formation by [PGE_2+adrenaline]. Experimental conditions were the same as for Figs. 5, 6 and 7.

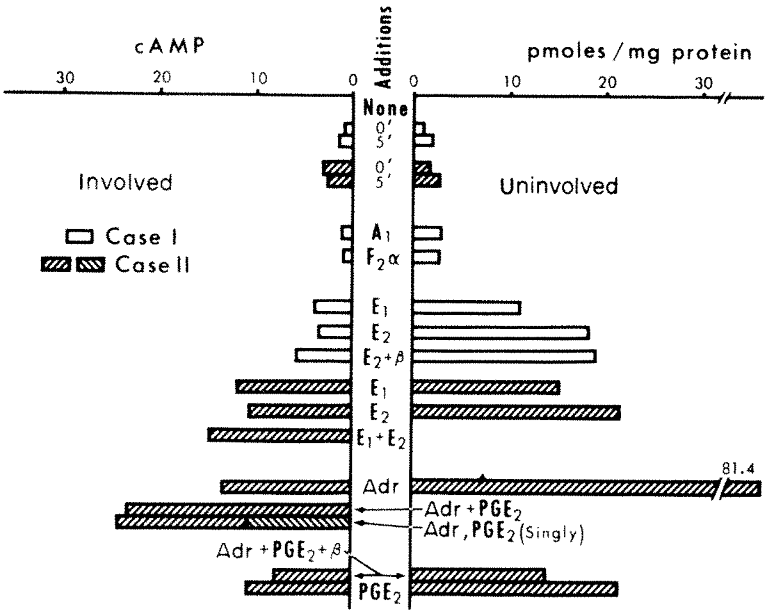

FIGURE 9. Effects of prostaglandins on cyclic AMP accumulation in involved and uninvolved epidermis taken from the same patients with psoriasis. Human epidermis was incubated for 5 min at 37°C with prostaglandins at a final concentration of 3×10^{-6}M. The β-blocker (β) used was propranolol at 10^{-4}M. Adrenaline (Adr) was added at 5×10^{-6}M. Cases I and II represent data from different patients.

of cyclic AMP in psoriatic epidermis almost equals the level reached with PGE_2 alone (Fig. 9, Case II). As in pig epidermis, the effects of PGE_2 and adrenaline on cyclic AMP accumulation in psoriatic epidermis are additive to the value obtained by a simultaneous addition of both drugs.

These data clearly indicate that, in psoriatic epidermis also, the site of action of PGE is different from the β-site where adrenaline works specifically. Thus the basic mode of action of prostaglandins on adenyl cyclase appears to be the same in human and pig epidermis. The degree of response appears to be more sluggish in the involved skin than in the uninvolved skin of patients with psoriasis.

General Comments

Although our conclusions on the effects and modes of action of prostaglandins on epidermal adenyl cyclase are generally substantiated by the data presented in this communication, there is one disturbing finding—the variability in response to the same concentration of PGE, the increase in cyclic AMP being from two to sevenfold for the same 3×10^{-6}M final concentration of PGE_2. Since no large variability is found within the same experiment, our analytical techniques appear to be reasonably reliable. This variability, then, may be partly due to a variable contamination of dermis taken by the keratome, and partly to individual variation, which may be difficult to avoid in experiments with animals. However, a third possibility, which we shall have to consider in the future, is the contribution from prostaglandins endogenously present in the epidermal slices. Just as ischaemia causes a rapid increase in cyclic AMP in epidermis (Yoshikawa *et al.*, 1975b), so damaged skin may also accumulate PGE.* This increase, which appears to be due to the sudden release of precursors for prostaglandins, peaks after about 20 min of injury.* (But cyclic AMP accumulation after ischaemia peaks at 5 min;

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*Personal communication with Dr V.A.Ziboh, University of Miami, School of Medicine, Miami, Florida.

Yoshikawa *et al.* (1975b). Thus, the ischaemic effect is probably not due to the increase in PGE. Since the content of PGE_2 in skin is in the order of nanograms per milligram skin,* its endogenous concentration is probably in the order of 10^{-6}M. This concentration as shown here is significant in regard to stimulation of epidermal adenyl cyclase. Therefore, the endogenous concentration and the possible change in PGE concentration after epidermal slicing and during the pre-incubation period have to be followed so that the experimental conditions can be standardized with respect to both endogenous PGE and cyclic AMP. For this reason further experiments on the effects of PGE on psoriatic epidermis in more patients were not carried out. Unlike adrenaline, where the quantitative difference between involved and uninvolved epidermis in regard to cyclic AMP formation is a major one, the PGE system in involved and uninvolved epidermis shows a relatively minor quantitative disturbance.

ACKNOWLEDGMENT

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Smooth muscle antibodies and alopecia areata

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SUMMARY

In seventy-eight patients with alopecia areata, the incidence of auto-antibodies to smooth muscle was greater than that in an age and sex matched control population.

Although the cause of alopecia areata is unknown there is evidence that it may belong to the group of auto-immune diseases. Muller & Winkelmann (1963) reported an increased incidence of thyroid disease in patients with alopecia areata and Morgans (1964) has shown alopecia areata to be more common in patients with thyrotoxicosis. A relationship between alopecia areata and vitiligo, and between vitiligo, thyroid disease, pernicious anaemia and diabetes mellitus has also been claimed by some authors (Cunliffe *et al.*, 1968). In addition Cunliffe *et al.* (1969) found the incidence of thyroid disease in patients with alopecia areata to be greater than in patients with psoriasis and controls; and an increased incidence of alopecia areata and diabetes mellitus was also found in the relatives of such patients. However, they found no significant difference in the incidence of thyroglobulin and thyroid complement fixing antibodies in patients with alopecia areata as compared with a 'general practice' population. More recently Kern *et al.*, 1973 have shown a significant correlation between alopecia areata and the presence of auto-antibodies to thyroglobulin, parietal, adrenal and thyroid cells.

It is a disease in which some 10–20% of cases have a family history (Sabouraud, 1929). Histologically there is an inflammatory condition with peri-bulbar and intrapapillary lymphohistiocytic infiltration and degeneration of the matrix cells (Thies, 1966). Fivaz (1953) demonstrated a lymphocytosis in approximately 70% of fifty-one clinically investigated patients with alopecia. In addition, corticosteroids are effective in bringing about regrowth of hair, albeit often temporarily, and presumably act by virtue of their anti-allergic and anti-inflammatory activity.

The present study was undertaken to determine the frequency of auto-antibodies in patients with alopecia areata as compared to an age and sex matched control population and to confirm the finding that there is a family history of known auto-immune disease in such patients and their families.

MATERIALS AND METHOD

Seventy-eight patients with alopecia areata were studied. The group consisted of an uninterrupted sequence of patients with this disease attending the outpatient department, the ages ranging from 10 to 68 years (mean 37·5 years). Forty-two patients were female and thirty-six were male. The age of onset ranged from 1 to 55 years (mean 26 years). The majority of the patients had moderately severe or severe alopecia including eight patients with total and universal alopecia.

The control group consisted of age/sex matched (± 2 years) hospital in-patients who had a variety of disorders that were not primarily auto-immune diseases (cardiovascular 17, respiratory 2, gastro-intestinal 23, renal disease 15, haematological 2, psychological 5 and miscellaneous 14).

The test group and control group were circularized and a personal or family history of asthma, hay fever, thyroid disease, diabetes, vitiligo, eczema and alopecia areata ascertained (Table 1). Serum auto-antibodies to nuclear factor (ANF), smooth muscle (SMA), mitochondria, thyroid, gastric parietal cells, adrenal, parathyroid and reticulin were sought using a sandwich indirect immunofluorescent technique (Beck, 1971).

TABLE 1. Personal and family history of various diseases in alopecia areata patients and age and sex matched controls

	Personal		Family	
	(1) Alopecia areata group	(2) Control group	(1) Alopecia areata group	(2) Control group
Asthma	4	0	11[b]	0
Hay fever	3	2	10	4
Hyperthyroidism	2	0	13	8
Hypothyroidism	0	0	4	3
Diabetes mellitus	1	1	16	13
Vitiligo	7[a]	1	2	1
Eczema	6	2	14	6
Alopecia areata	–	2	20[c]	4

(1) 78 replies; (2) 53 replies. a) $P<0·08$; b) $P<0·002$; c) $P<0·005$.

RESULTS

The personal and family history of various diseases in alopecia areata patients and their age and sex matched controls is shown in Table 1. Although there are apparent increases in the frequency of most diseases in the alopecia patients as compared with that found in the controls, only in the case of vitiligo does this approach significance ($P<0·08$). There are also apparent increases in the frequency of all diseases within the families of alopecia patients as compared with the control families but only in the case of asthma ($P<0·002$) and alopecia areata ($P<0·005$) are these significant.

Table 2 shows the frequency, immunoglobulin and titre of non-organ and organ specific auto-antibodies in alopecia patients and controls. A significant increase ($P<0·05$) in the incidence of both organ specific and non-organ specific auto-antibodies is seen in the alopecia patients as compared with controls. If, however, the organ specific and non-organ specific auto-antibody results are considered individually the differences between alopecia and control groups are not significant.

The most striking increase in auto-antibodies is in the smooth muscle antibodies (SMA). A four-fold significant increase ($P<0·01$) in SMA is noted in the alopecia group of patients. All other individual auto-antibody differences between test and control groups fall short of statistical significance. Table 2 also shows that most of the auto-antibodies found belong to the IgG class and are of low titre.

TABLE 2. The immunoglobulin classes and titres of serum autoantibodies found in seventy-eight alopecia areata patients and seventy eight age and sex matched controls

Type of autoantibody	Incidence of auto-antibodies	Immunoglobulin classes			Serial dilution					
		IgG	IgA	IgM	neat	1/4	1/16	1/64	1/256	1/1024
Smooth muscle	16 (4)[a]	14(4)	—	3(1)	—	—	4(1)	8(2)	4(0)	0(1)
Antinuclear	0 (2)	0(2)	—	—	—	—	0(2)	—	—	—
Antimichondrial	0 (2)*	—	—	—	—	—	—	—	—	—
Thyroid (antimicrosomal)	13 (6)[b]	12(6)	0(1)	5(0)	3(1)	5(3)	3(2)	2(0)	—	—
Gastric (antiparietal cell)	6 (5)	6(5)	0(1)	3(4)	2(0)	0(1)	0(3)	2(1)	2(0)	—
Adrenal	0 (2)*	0(1)	—	—	—	(0)1	0(1)	—	—	—
Parathyroid	0 (1)	0(1)	—	—	—	1(0)	—	—	—	—
Reticulin	0 (1)*									
Total	35 (23)[c]									

Control figures are given in brackets.

a) $P < 0{\cdot}02$; b) $P < 0{\cdot}3$; c) $p < 0{\cdot}05$.

* Insufficient serum to determine immunoglobulin class and titre.

DISCUSSION

In our series no significant increase in the incidence of thyroid disease in the alopecia areata patients was noted although the association of thyroid disease and alopecia areata has previously been recorded (Morgans, 1964; Cunliffe *et al.*, 1969; Muller, & Winkelmann, 1963). A significant increase in the number of relatives with asthma was found in the alopecia group compared with the controls and does not appear to have been recorded previously. The association of alopecia areata and vitiligo and a significant family history of alopecia areata was found in the disease group in agreement with Cunliffe *et al.*, 1968.

In this present study we have been unable to substantiate the findings of Kern *et al.*, 1973 who showed a statistically significant increase in antibodies against thyroglobulin, parietal, adrenal and thyroid cells in patients with alopecia areata but our results are in agreement with those of Cunliffe *et al.* (1969) who found no significant difference in the incidence of thyroglobulin and thyroid complement fixing antibodies in patients with alopecia areata.

Of considerable interest is our finding of a significant increase in smooth muscle antibodies. These were first reported in the sera of patients with chronic active hepatitis (Johnson, Holborow & Glynn, 1965). They were said to be indicative of the auto-immune forms of hepatitis and shown to be mainly IgG antibodies (Doniach & Walker, 1969). More recently they have been shown to be present in acute hepatitis, occurring mainly as IgM (Farrow *et al.*, 1970), in malignant disease (Whitehouse & Holborow, 1971) and in infectious mononucleosis (Holborow *et al.*, 1973). Holborow, Hemsted & Mead (1973) showed that a high percentage of patients (81% of 126 patients) over 10 years of age with sero-positive infectious mononucleosis had SMA when tested within 1 month of onset. Among forty-five adults with past histories of sero-positive infectious mononucleosis more than 1 year before the incidence of SMA was 33% in contrast to 14% in ninety-eight subjects with a negative history for infectious mononucleosis. The percentage of SMA in this control series seems much higher than the present survey. This difference might in part be explained by a difference in technique, all our sera were tested at 1 in 16, their at 1 in 10.

However, the incidence of SMA in our control patients (5·1%) is high in comparison to that found by Hooper *et al.* (1972) in a study of a large normal population in Australia, the incidence (over 20–70 years) ranging from only 1·1 to 2·2%. In addition these workers have also found that, unlike auto-antibodies to nuclei, thyroid epithelial cells and gastric parietal cells, SMA were not more prevalent in females nor did they increase with age. Correspondingly they suggested that, unlike age–sex dependent auto-antibodies, SMA might be influenced by extrinsic factors such as infection. The control group in our series is biased to some extent since we chose patients who were not primarily suffering from overt auto-immune disease (lupus erythematosus, scleroderma, rheumatoid arthritis, thyrotoxicosis, Hashimoto's disease, myxoedema, pernicious anaemia, primary biliary cirrhosis and chronic active hepatitis). By making such a choice it might be argued that SMA could be present in greater numbers in association with these diseases but there is no evidence to suggest that this is so except in the case of liver disease.

To determine why subjects with a negative history for infectious mononucleosis show an appreciable incidence of SMA, Holborow *et al.* (1973) tested a group for E.B. virus antibody and for SMA but no correlation between the two was evident. It has been suggested there might be other virus infections that can give rise to SMA and it has been reported by Ajdukiewicz *et al.*, 1972 that cytomegalovirus may do so. Hepatic damage does occur in infectious mononucleosis and must be considered as a possible factor leading to increased SMA.

Alopecia areata is of unknown cause and so far attempts to demonstrate an infective agent have been unsuccessful. Viral infection could explain the increase in SMA, possibly by direct infection of the arrector pili muscles of involved hair follicles. Confirmation of this suggestion, however, must await further studies.

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Serum anti-tumour antibodies and auto-antibodies in vitiligo

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SUMMARY

Anti-tumour antibody was sought in the sera of three groups of patients; idiopathic vitiligo, malignant melanoma and miscellaneous skin disorders. No precipitating antibody to a series of prepared pigmented tumour antigens was demonstrated. All sera were tested for a number of auto-antibodies. No significant differences were detected between the groups. The evidence for an immunological factor in the causation of vitiligo remains circumstantial only.

The evidence that vitiligo is a member of the family of organ-specific auto-immune disease (*Lancet*, 1971) has been found in its association with systemic and cutaneous disorders currently considered to be of auto-immune aetiology (Cunliffe *et al.*, 1968; Dawber, 1968, 1970; Grunnet *et al.*, 1970; Howitz & Schwartz, 1971). There is also an increased incidence of organ-specific auto-antibodies in vitiligo patients (Ochi & DeGroot, 1969; Brostoff, Bor & Feiwel, 1969; Bor, Feiwel & Chanarin, 1969). More direct support for the auto-immune hypothesis was provided by Langhof, Feuerstein & Schabinski (1965) who demonstrated precipitating 'anti-melanin antibody' in the sera of the majority of twenty-six vitiligo patients. In this study, we have attempted to confirm the work of Langhof *et al.* (1965), and in addition have screened the sera from forty-two patients with vitiligo for a variety of auto-antibodies.

PATIENTS AND METHODS

The age and sex distributions of the patients studied are summarized in Table 1. All were of European descent. In the vitiligo group, the duration of the disorder ranged from 6 weeks to 30 years and its extent was unrelated to an early or late onset. There was a wide spectrum of activity which extended from almost complete spontaneous repigmentation to rapid progressive depigmentation. The control population, age and sex matched, comprised a group with a variety of skin diseases in which immune

TABLE 1. Results of auto-antibody tests

	Vitiligo (42 cases)	Miscellaneous skin disorders (controls) (42 cases)	Malignant melanoma (17 cases)
Mean Age	33·1	32·8	48 9
Range (years)	5–67	7–61	22–71
Sex Female	28	28	10
Male	14	14	7
Thyroglobulin precipitin	1–+++	0	0
Thyroglobulin TRC (titre)	2–1/16 1–1/4000	0	0
Thyroid microsomal antibody	3–+ 2–++	1–++ 1–+++	0
Gastric parietal cell antibody	2–+ 2–++ 2–+++	1–+ 2–++ 5–+++	1–+
Anti-nuclear factor (titre)	4–1/16 1–1/16 HN 1–1/64 HN	5–1/16 1–1/64 HN 1–1/256 HNS	2–1/16
Rheumatoid factor—rheumaton (titre)	3–+ 1–++ 4–+++	1–1/16 2–++	2–1/1 2–++
Mitochondrial antibody	0	2–+	0
Smooth muscle antibody	2–+ 2–++	4–+	1–+
Salivary duct antibody	1–+	1–+	0
Adrenal cortex antibody	0	0	0
Positive results Female	30	16	3
Male	4	11	5
Total	34	27	8

+, weakly positive; ++, positive; +++, strongly positive; H, homogenous pattern; N, nucleolar pattern; S, speckled pattern; O, tested but negative.

mechanisms have not been invoked, *viz.* psoriasis (20), verruca vulgaris(5), acne vulgaris (4), rosacea, eczema reactions and stasis ulcer (3 each), pityriasis capitis, milia, paronychia and solar keratoses (1 each). All the patients with malignant melanoma had histological confirmation of the disease and had been treated by surgical resection of their primary lesions only.

Preparation of tumour extracted antigens

Melanoma antigen was prepared from two primary melanocarcinomas (Ag. 1 and Ag. 2) and from a lentigo maligna (Ag. 3) showing early malignant change. Similar antigenic preparations were also made from a lentigo simplex and from a benign blue naevus (Ag. 4 and Ag. 5 respectively). Tumour samples obtained at operation from five different individuals were placed in sterile containers after division of the specimens for histological examination. All lesions were confirmed histologically but we were unable to study the ultrastructure of the specimens. Fat and fibrous tissue were dissected away and the specimens finely chopped with a scalpel blade in Eagle's Minimal Essential Medium containing penicillin and streptomycin. The materials were then homogenized in an M.S.E. blade homogenizer and thereafter spun for 30 min at 1500 rev/min at 4°C (Mackie *et al.*, 1972). The supernatants were separated and the protein concentration of each was estimated by spectrophotometry. These were as follows: Ag.1, 14·5; Ag.2, 9·8; Ag.3, 1·4; Ag.4, 11·0; Ag.5, 0·8 μg/ml.

Precipitation testing for antibodies

In initial studies, sera from twenty vitiligo patients, twenty controls and seventeen patients with malignant melanoma were tested against undiluted Ag. 1, using a double diffusion Ouchterlony technique. Subsequently, and again using a double diffusion Ouchterlony technique, sera from twelve patients with vitiligo, twelve controls, and six patients with malignant melanoma were screened undiluted against Ags. 2, 3 and 4 undiluted and at $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, and $\frac{1}{64}$ dilution in saline.

Serum auto-antibodies

Standard indirect immunofluorescent techniques were used to detect antinuclear factor (Beck, 1961), antithyroglobulin antibody (Fulthorpe *et al.*, 1961), thyroid microsomal antibody (Holborow *et al.*, 1959), gastric parietal cell antibody (Adams *et al.*, 1964), antimitochondrial antibody (Goudie, MacSween & Goldberg, 1966), smooth muscle antibody (Johnson, Holborow & Glynn, 1965), adrenal cortex antibody (Goudie *et al.*, 1969), and salivary duct antibody (MacSween *et al.*, 1967). Sera were screened for rheumatoid factor using the 'Rheumaton' test (Warner Laboratories) and a quantitative assessment of positive sera was made by the R3 test (Warner Laboratories).

RESULTS

Anti-tumour antibodies. In no instance was precipitating antibody to any of the pigmented tumour extracted antigens demonstrated.

Auto-antibodies. The auto-antibody results are summarized in Table 1. No statistically significant differences emerged between the different groups.

DISCUSSION

In the present study, we have been unable to confirm the finding of Langhof *et al.* (1965) of circulating precipitating antibody to 'melanin' in the sera of vitiligo patients. The 'melanoprotein' tumour antigens we used were prepared in a similar way to the antigen which Langhof *et al.* (1965) tested and which they prepared from metastatic melanocarcinoma tissue. It may be that melanoma tumour antigen is not an appropriate one to use as the antigens produced by malignant cells may be different from those produced by normal melanocytes, the absence of which constitutes the histological hallmark of vitiligo (Fitzpatrick, *et al.*, 1971). In an attempt to obviate this possibility, antigen preparations were also obtained for the present study from a lentigo simplex and from a benign blue naevus. However, only a partial attempt was made to characterize the nature of these antigens and it is evident that the prepared saline extracts must have contained a heterogeneous antigen mixture. Ag. 1 was used to immunize rabbits and, following absorption with human liver, kidney and lung homogenates, it was found that the rabbit antiserum produced precipitin lines with Ag. 1 and Ag. 2 which showed a reaction of identity. No precipitin lines were obtained with the antiserum against Ag. 5. Precipitin lines were produced with the antiserum against Ag. 3 and Ag. 4 but these showed reaction of non-identity with Ag. 1. and Ag. 2. It may be that these results do indicate antigenic differences between malignant and benign pigmented lesions but clearly further work on the characterization of such antigens is indicated. No evidence, however, was found of precipitating antibodies in the vitiligo patients' sera against any of the antigens used.

Neither were circulating precipitating antibodies demonstrable in the sera from our melanoma patients. Circulating antibodies against malignant melanoma cell cytoplasm have been found in patients with malignant melanomas using an immunofluorescent technique (Lewis *et al.*, 1969). Recently Copeman *et al.* (1973) reported similar findings in fifteen patients with resolving halo naevi; lesions which have an association with vitiligo (Lerner, 1971) and from which two of our vitiligo patients

TABLE 2. Comparative results of auto-antibody studies in vitiligo

	Cunliffe *et al.* (1968)		Bor *et al.* (1969)		Brostoff *et al.* (1969)		Howitz & Schwartz (1971)		Woolfson *et al.* (1974)	
	A	B	A	B	A	B	A	B	A	B
No. of patients	52	52	62	62	80	80	102	102	42	42
Thyroglobulin precipitin	—	—	—	—	—	—	—	—	2·3	0
Thyroglobulin TRC	24·5	4·0	—	—	8 7	0	—	—	7 1	0
Thyroglobulin microsomal antibody	12·0	0	—	—	27·5	7·5	—	—	11 9	4 7
Gastric parietal cell antibody	—	—	22·5	8·0	21·2	1·2	20·0	6·0	14·2	19·0
Anti nuclear factor	—	—	—	—	3·7	2·5	—	—	14·2	16 6
Rheumatoid factor —rheumaton	—	—	—	—	—	—	—	—	19·0	7·1
Mitochondrial antibody	—	—	—	—	—	—	—	—	0	4·7
Smooth muscle antibody	—	—	—	—	0	0	—	—	9·5	9 5
Salivary duct antibody	—	—	—	—	0	0	—	—	2·3	2 3
Adrenal cortex antibody	—	—	—	—	4·0	0	—	—	0	0

Results expressed as percentages; 0, tested but negative; —, not tested; A, Vitiligo; B, Controls;

also suffered. However, Copeman *et al.* (1973) did not detect this anticytoplasmic antibody in forty-seven patients with vitiligo unassociated with halo naevi. Thus neither in the studies of Copeman *et al.* (1973) nor in the present study has any antibody peculiar to vitiligo been confirmed. It seems that until anti-melanin or anti-melanocyte antibody has been clearly demonstrated the evidence for an immune aetiology for vitiligo must remain at best indirect.

The percentage incidence of serum auto-antibodies in the present study are compared in Table 2 with results in previously published series. The number of positive results obtained in our series is small and in particular there was no marked increase in positive results in the vitiligo group. All series show that less than 30% of patients with vitiligo have some circulating auto-antibody. It has been suggested that vitiligo patients with serum auto-antibodies present may be of a different aetiological type, and also that vitiligo associated with halo naevi may form a further sub-group. The majority of patients with vitiligo do not, however, have these co-existent findings. If an immune process is involved in idiopathic vitiligo, it seems probable that it is confined to the integument and not reflected systemically. This would not be surprising for in vitiligo only the melanocytes of the skin, mucous membrane and hair are affected; those of the retina, uveal tracts, leptomeninges and inner ear escape destruction (Fitzpatrick *et al.*, 1971).

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The modifying effect of autologous serum on leukocyte migration inhibition by liver antigens in systemic lupus erythematosus

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SUMMARY

The leukocyte migration test was used to examine patients with systemic lupus erythematosus (SLE) for evidence of cell-mediated immunity to human liver microsomes and mitochondria. Testing of both these antigens with leukocytes from patients was carried out simultaneously in culture medium enriched by either 20% fetal bovine serum (the standard test) or substituted 20% autologous serum. Normal controls showed little or no reactivity in the standard test whereas the SLE group revealed a high incidence of significant leukocyte migration inhibition to both antigens which, however, was considerably reduced by the substitution of autologous serum.

The results suggest that the sera of patients with SLE contain factor(s) capable of blocking the *in vitro* expression of cell-mediated immunity to human tissue antigens. These factor(s), if active *in vivo*, might have a role in controlling some of the auto-immune manifestations of the disease.

Although the aetiology of systemic lupus erythematosus (SLE) remains uncertain, auto-immune phenomena are a prominent feature of the disorder (Holman, 1965). While auto-antibodies may be concerned in the production of some of the lesions (Koffler, Schur & Kunkel, 1967) it is also possible that cell-mediated immunity may be involved, as the development of an experimental auto-immune lesion has been shown to require as a dual necessity the presence of both cell-mediated and humoral immune responses (Brown, Glynn & Holborow, 1967). The leukocyte migration test has been shown to be an *in vitro* indicator of cell-mediated immunity (Søborg & Bendixen, 1967; Federlin *et al.*, 1971) and in earlier studies has produced evidence of cellular sensitization to tissue antigens in Addison's disease (Nerup, Andersen & Bendixen, 1970), Sjögren's syndrome (Berry, Bacon & Davis, 1972), Hashimoto's disease (Calder *et al.*, 1972), chronic active hepatitis and primary biliary cirrhosis (Miller *et al.*, 1972), progressive systemic sclerosis (Hughes, Holt & Rowell, 1974a) and SLE (Hughes, Holt & Rowell, 1974b).

The present report describes further investigations in SLE in the course of which the leukocyte migration inhibition to cellular sub-fractions of human liver was modified (or 'blocked') by the addition of autologous serum. This 'blocking' of cell-mediated hypersensitivity to tissue antigens, if active *in vivo*, might account for the generally rather poor correlation that we have noted previously between the activity of the disease in SLE and the extent of leukocyte migration inhibition (Hughes *et al.*, 1974b).

MATERIALS AND METHODS

Patients

Sixteen patients with SLE (Medical Research Council, 1961), one of whom was male, with a mean age of 40·6 ± 15·2 years, were studied. Eleven members of the group met at least four of the American Rheumatism Association criteria for the classification of the disease (Cohen *et al.*, 1971), while all of the remaining five patients were positive for three of the criteria. The duration of disease ranged from 2 to 23 years, with five of the patients having been under regular outpatient review for 10 years or more. Two of the group were not receiving steroid treatment at the time of the tests, while in the remainder prednisone dosage ranged from 2·5 to 20 mg per day. Controls consisted of nineteen healthy volunteers, one of whom was male, with a mean age of 36·9 ± 13·1 years.

Human liver microsomes and mitochondria

These antigens were freshly prepared from human cadaver liver obtained within 3 h of death as previously described (Hughes *et al.*, 1974a).

The leukocyte migration test

The technique reported previously (Hughes *et al.*, 1974a) was modified so that testing of the two liver antigens with cells from patients with SLE was now carried out simultaneously in a culture medium (TC199; Wellcome Reagents Ltd.) enriched either by 20% fetal bovine serum (Wellcome Reagents Ltd.)—the standard form of the test—or by substituted 20% autologous serum.

Statistical methods

Since the 'migration index' is a ratio, logarithms of the observations used to derive this ratio were used in a Student's *t*-test in the statistical analysis of the experimental results.

RESULTS

Preliminary experiments

These were performed by the standard test in a small group of controls with varying doses of both human liver microsomes and mitochondria. It was found that 175 μg/ml and 200 μg/ml respectively of these two antigens produced leukocyte migration inhibition that fell within the 'normal' range of 0·80–1·20. These concentrations were, therefore, used to examine both the patients and a larger control group and the results are shown in Figs. 1 and 2.

The fetal bovine serum test

When tested with human liver microsomes in fetal bovine serum, the SLE group showed a significant degree of migration inhibition (mean migration index 0·58 ± 0·13) when compared with the normal controls (mean migration index 0·93 ± 0·16; $t = 6·9$; $P < 0·001$). Fifteen of the sixteen patients showed sensitization with migration indices that fell below 0·80, whereas only four controls showed what was always a minimal degree of migration inhibition. Testing with human liver mitochondria in fetal

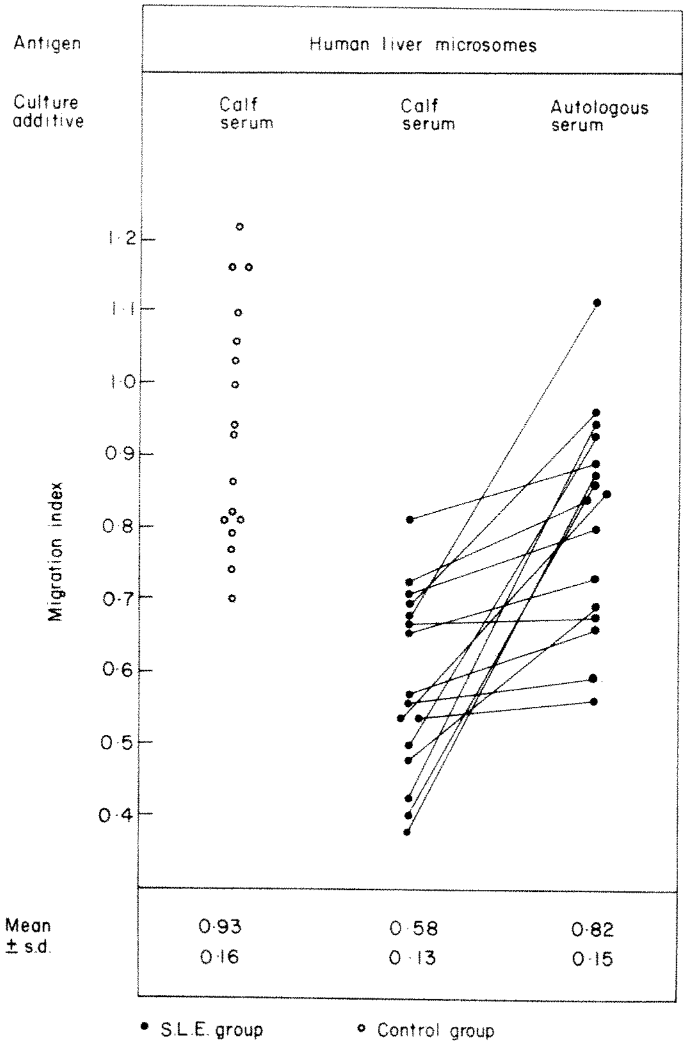

FIGURE 1. Leukocyte migration inhibition to human liver microsomes (175 μgm/ml) in controls and patients with SLE tested in fetal bovine and autologous serum.

bovine serum produced similar results, the patients with SLE showing a significant degree of migration inhibition (mean migration index 0·64 ± 0·14) when compared with the normal response of the control group (mean migration index 0·91 ± 0·10; $t = 4·3$; $P < 0·001$). Ten of the twelve patients had individual migration indices that fell below 0·80, whereas only two of the normal controls showed what again was only a minimal degree of reactivity.

The autologous serum test

The substitution of autologous for fetal bovine serum had, however, an appreciable modifying effect on the resulting migration indices of many of the patients with SLE. With both antigens there was a diminution in the mean leukocyte migration inhibition with a reduction, in the case of the liver microsomes, from 0·58 ± 0·13 to 0·82 ± 0·15 ($t = 4·6$; $P < 0·001$) and, in the case of the liver mitochon-

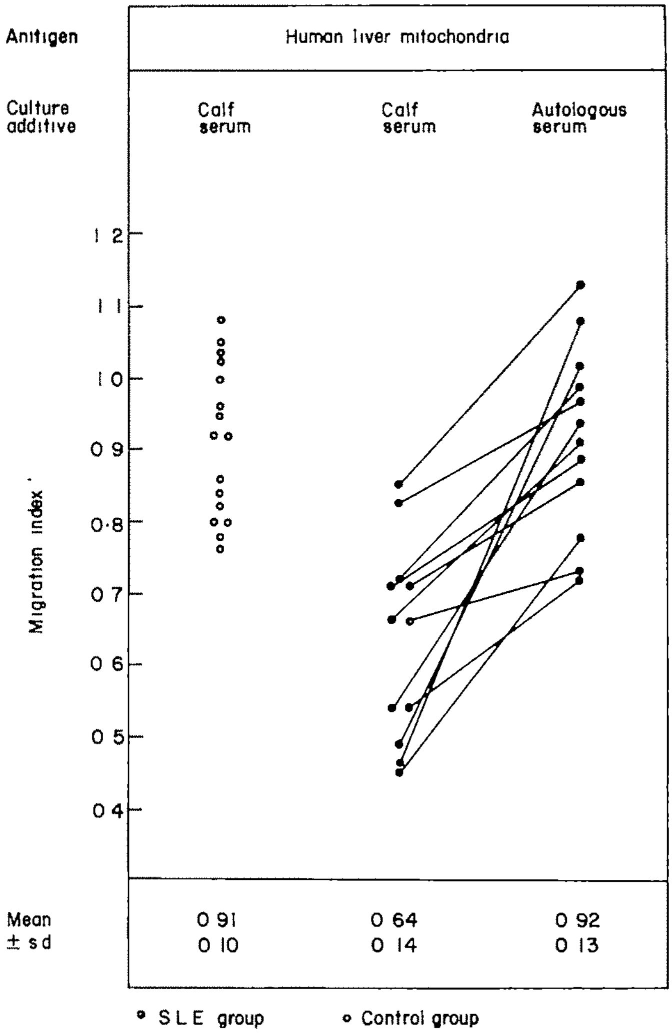

FIGURE 2. Leukocyte migration inhibition to human liver mitochondria (200 μgm/ml) in controls and patients with SLE tested in fetal bovine and autologous serum.

dria, from 0·64 ± 0·14 to 0·92 ± 0·13 ($t = 5·4$; $P < 0·001$). This modifying effect was more marked in some individuals than others while in a few there seemed to be little or no effect at all. Thus, of the fifteen patients showing sensitivity to microsomes in the standard test, nine showed sufficient reduction of migration inhibition in autologous serum for their migration indices to return to 0·80 or more.

The pattern of response to mitochondria in autologous serum was also very similar. Of the ten patients showing sensitization to this antigen in the standard test, seven showed a return of their individual migration indices to above 0·80 when tested in autologous serum.

DISCUSSION

The present investigation, which includes previously untested patients and controls, provides additional confirmation of our earlier report of leukocyte migration inhibition in SLE by human tissue antigens (Hughes *et al.*, 1974b). The use of freshly prepared human liver microsomes and mitochondria, at even lower concentrations than were usual formerly, has once more produced a significant degree of migration inhibition in patients with SLE. The principal observation, however, is that this *in vitro* evidence of cellular sensitization to human tissue antigens can be appreciably modified, i.e. reduced in many of the patients by the substitution of autologous serum for the fetal bovine serum of the standard leukocyte migration test. This *in vitro* reduction of migration inhibition tended to occur equally with both the liver antigens and the pattern of response is very suggestive of the presence of 'blocking' or 'inhibiting' serum factors.

The exact significance of leukocyte migration inhibition in response to tissue antigens in terms of the pathogenesis of human disease remains uncertain. The phenomenon can develop as a secondary manifestation of non auto-immune tissue damage (Wartenberg & Brostoff, 1973) and, in these circumstances, is possibly analogous to the usually transient and low titre auto-antibody response that also occurs after tissue trauma (Hutchinson, Patel & McArthur, 1971). However, evidence from both human 'auto-immune' diseases (Nerup *et al.*, 1970; Miller *et al.*, 1972) and proven auto-immune experimental models (Krohn & Finlayson, 1973) does suggest that the test may also reflect pathogenetically important cell-mediated auto-immunity. SLE has, as a striking feature, a high incidence of both auto-antibodies (Beck, 1963) and leukocyte migration inhibition to a wide range of tissue antigens (Hughes *et al.*, 1974b). We have suggested that this spectrum of cellular sensitization by presumptive thymus-dependent (T) lymphocytes is more in favour of a primary loss of tolerance, facilitating perhaps both auto-antibody production (Allison, Denman & Barnes, 1971) and lymphocyte cytotoxicity (Trayonova, Sura & Svet-Moldavsky, 1966), than a secondary phenomenon consequent to earlier episodes of tissue damage and inflammation. However, throughout our investigations in SLE there has been no firm correlation between any *in vitro* evidence of cellular sensitization to tissue antigens and either disease activity or the pattern of organ involvement. A possible explanation for this lack of correlation could be that the cellular sensitization was merely a secondary phenomenon, as outlined above.

Nevertheless, our finding that autologous serum often diminishes the *in vitro* expression of such cellular sensitization raises the alternative possibility that serum from patients with SLE contains factors capable of 'blocking' what would otherwise be pathogenetically important cell-mediated auto-sensitization. Although in an earlier study of SLE we did not find any correlation between anti-mitochondrial antibodies and leukocyte migration inhibition with mitochondria (Hughes *et al.*, 1974b), such a serum factor could be either a 'blocking' auto-antibody, analogous to that described in skin allografted mice by Micklem (1971), or an immunosuppressive alpha-globulin of the type described and characterized by Mowbray & Hargrave (1966), Milton (1971) and Glazer, Ofek & Nelken (1972). As a 'blocking' auto-antibody acts by masking antigenic determinants and an immunosuppressive alpha-globulin may antagonize the antigen recognition mechanism of lymphocytes (Milton, 1971), it is distinctly possible that the two processes could act synergistically *in vivo* to control the ill-effects of cell-mediated auto-immunity. Further investigations in SLE, therefore, may well reveal the existence of a delicate balance between auto-aggression and attempts at homoeostasis.

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Emotions and skin (II)—the conditioning of scratch responses in cases of lichen simplex

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SUMMARY

Lichen simplex is generally regarded as a condition initiated and perpetuated by scratching and emotional tension. It was felt that the scratching might partly be a conditional response to itching and other signals, and that feelings of guilt, anxiety and hostility would be prominent features in these patients. Conditioning experiments designed to establish scratch responses to an itch stimulus (UCS) and a tone (CS) showed that lichen simplex patients conditioned more readily and extinguished more slowly than controls. These findings were more marked when the itch stimulus was applied to affected as compared with normal skin. It was not possible to distinguish differences in the psychological tests between patients and controls. The possible significance of these findings is discussed.

Lichen simplex chronicus, described by Vidal in 1883, is generally regarded as being caused by persistent scratching in response to some kind of emotional tension. As Rook, Wilkinson & Ebling state (1972), 'Lichenification describes a pattern of response of the predisposed skin to repeated rubbing.' In some instances a minor trauma, infection, or other skin lesion of an itchy kind, acts as the trigger to continuous scratching, resulting in the characteristic reddened, pigmented, scaly, thickened and sharply demarcated patch of skin, with exaggerated markings traversing its extent. Itching is the principal complaint and scratching the invariable response. Consequently, the lesions are situated on skin easily accessible to the fingers and particularly on the nape of the neck, the arms, legs and thighs and the anogenital regions.

The neuropathology of the skin was fully described by Cowan (1964) who demonstrated in ten cases a proliferation of fine non-myelinated nerve fibres in the upper dermis. Sinclair (1973) also noted the increase in nerve endings and Schwann cells associated with an increased 'turnover' of neural elements, along with epidermal thickening. Cowan was of the opinion that the extensive disorganization of cutaneous nerve endings could be an explanation of the itchy hyper-excitability which is partly the cause of the intense and repeated scratching. Presumably the neuro-histopathological changes are the response to itching and scratching in the first place, as there seems to be no evidence that such changes are primary causes of itching in vulnerable areas of skin. However, it is generally agreed that some patients with lichen simplex have a dry 'atopic' skin, which may make them particularly susceptible to the disorder.

Many authors have commented on the relationship of emotional tension to the scratching which is an essential factor in the production of the lesions (Allerhand, Gough & Gvais, 1950; Cormia, 1951; Obermeyer, 1955; Rook *et al.*, 1972). Obermeyer is rightly critical of some psychoanalytical speculations relating the scratching to repressed sexual drives. Other authors (Pilsbury, 1953) have noted the tendency of patients with neurodermatitis to scratch when hostile feelings are aroused. An earlier paper (Jordan & Whitlock, 1972) appeared to show that patients with atopic dermatitis scored more on measures of hostility than matched controls but a more recent assessment of this factor (Jordan & Whitlock, 1974) related the hostility to anxiety rather than to the skin disorder as such. The localized nature of lichen simplex and its possible association with atopic dermatitis and other forms of neurodermatitis suggest that patients with lichen simplex might also exhibit some of the psychological and psycho-physiological features manifested by patients with these other disorders. Bearing in mind that in atopic dermatitis the lesions are widely distributed whereas those of lichen simplex are sharply localized, it was anticipated that conditioned scratch responses might be more readily elicited when the stimulus was applied to the lesion, compared with the normal skin. We also postulated that feelings of guilt, anxiety and hostility would particularly elicit scratch responses in the 'conditioned' area and that patients with lichen simplex would score more highly on these variables than matched controls.

MATERIALS AND METHODS

The methods of psychological and experimental testing were essentially the same as those used in the earlier study (Jordan & Whitlock, 1972). Significant differences were modifications to the apparatus to conform with the standard specifications of the constant current electrode system recommended by Lader & Wing (1966) and the incorporation of a specially constructed Field Effect Transistor balance bridge and direct reading millivolt meter into the recording circuit. Calibrated resistors were built in and could be substituted for the patient to check equipment operation and span control setting on the graph record and thus enabled us to validate quantitative graphic measurements.

The patients

Eleven patients with lesions of lichen simplex chronicus were examined, five males and six females with ages ranging from 18–69 years, the mean age being 50·5 years. The lesions, often multiple, were distributed predominantly on the lower limbs (ten cases). There was one case of anogenital lichen simplex and rather surprisingly, only one with the classical lesion on the nape of the neck. Patches of lichen simplex had been present on average for 8·3 years, with a range from 6 months to 30 years. This last case had fortunately experienced a remission period of 10 years before the present relapse.

The controls

The controls, matched for age and sex with the index cases, were drawn from patients attending a dermatological clinic. Four males and four females were receiving treatment for solar keratoses, one male had a healing tinea cruris and one female a healed attack of pityriasis rosea. The other female control had chronic psoriasis.

Psychological investigations

We used the same psychological tests described in the earlier paper. All patients were interviewed and a full psychological and social history was obtained, in addition to details of their dermatological disorders.

Experimental techniques

The conditioning experiments were conducted in a sound-reduced temperature-controlled laboratory (22°C ± 1°C). The experiments were conducted in five parts.

(1) Determination of itch threshold (the unconditioned stimulus).
(2) Presentation of the unconditioned stimulus (habituation).
(3) Presentation of the conditioned stimulus (a tone).
(4) Conditioning.
(5) Extinction.

The experiments were carried out with the concentric itch stimulus electrodes applied at a standard site over the anterior aspect of the wrist. In addition, in six patients conditioning and extinction were carried out with the stimulus electrodes placed over lesions of lichen simplex as well as over normal skin. All patients were tested at the same time of day (15·00 h) and hydration was maintained by giving one or two cups of tea or other liquid as desired 45 min before testing.

The conditioned stimulus (CS) was produced by a variable pitch and intensity audio-activator, the tone being delivered to the patient by comfortable light-weight headphones. The itch sensation stimulus and audio system relay closure were indicated by marker systems on the recorder, to provide graphic records of both events, their initiation and duration.

RESULTS

Psychometric tests

Apart from significant differences ($P < \cdot 05$) in the Anxiety Scales of the M.M.P.I. and the Introversion Scale of the 16 P.F., there were no other features in the psychometric testing that distinguished controls from patients. In fact, the experimental group emerged as less anxious but more introverted than the controls, a finding which could not be explained in terms of repression or denial as measured by the Lie Scale of the E.P.I. and the Repression Scale on the M.M.P.I. (Welsh & Dahlstrom, 1956).

Psycho-physiological testing

The results of the conditioning and related experiments are set out in the Table 1.

Itch thresholds

These varied considerably both between patients and controls and between affected and unaffected skin in the patients. However, there were no constant differences between patients and controls and although some patients showed lower itch thresholds on the affected areas of skin, this was by no means a constant finding.

DISCUSSION

The absence of major psychometric differences between patients and controls did not support the assumption that patients with lichen simplex would manifest greater feelings of guilt, anxiety and hostility. In fact, the higher measure of anxiety in the controls suggested that this affect was relatively absent in the patients. An examination of the developmental histories and current sources of tension in both groups showed that at the time of testing domestic and other worries were, if anything, more prominent in the lives of the controls, although it would be impossible in the lichen simplex group to relate such tension to the skin condition. On the other hand, nine of the eleven lichen simplex patients stated that emotional tension was related to scratching and itching, a finding common enough in

TABLE 1. Experimental results

Normal skin $n = 11$	Patients	Controls	Statistical Significance (P)
1. No. of trials required for habituation to unconditioned stimulus (itch stimulus)	4·9	4·4	N.S.
2. No. of trials of habituation to conditioned stimulus (tone)	6·3	6·2	N.S.
3. No. of conditioned scratch responses	1·8	0·2	<0·01
4. No. of conditioned G.S.R.s	5·6	3·5	N.S.
5. Extinction phase. No. of conditioned scratch responses	0·4	0·3	N.S.
6. Extinction phase. No. of conditioned G.S.Rs.	3·5	3·3	N.S.
Lichenified skin $n = 6$			
1. No. of conditioned scratch responses	5	0·0	<0·001
2. No. of conditioned G.S.Rs.	6·3	3·7	N.S.
3. Extinction phase. No. of conditioned scratch responses	2·3	0·2	<0·01
4. Extinction phase. No. of conditioned G.S.Rs.	4·1	2·3	N.S.

G.S.R., Galvanic skin response.

many pruritic dermatoses. Possibly our own psychometric findings may have been affected by the greater incidence of domestic and other worries in the lives of the controls, compared with the patient, at the time of testing. Nevertheless, one part of our original hypothesis was not supported by our findings.

However, as predicted, the greater frequency with which conditioned scratch responses were elicited in the lichen simplex patients, a frequency which became even more significant when the unconditioned stimulus was applied to the skin lesion rather than to the normal skin, strongly suggests that a pattern of scratching has developed in these patients which has additionally a relative specificity for certain areas of skin. Further support for this opinion is derived from the greater number of scratch responses during the extinction phase, when the unconditioned stimulus was applied to patches of lichen simplex. The larger number of conditioned G.S.Rs, both during conditioning and extinction, in the lichen simplex patients compared with controls, although not statistically significant, suggested that stimuli to the lichenified skin had greater arousal potential than the same stimuli applied to normal skin.

Our findings therefore support the clinical observation that lichen simplex sufferers are chronic scratchers and that resort to this behaviour may be a learned response which has now become a fixed pattern, possibly when affective disturbance in the shape of emotional tension troubles the patient. The possibility that these findings were influenced by the higher introversion score in the experimental group (see Franks, 1956) was checked by correlating the scores with the frequency of conditioned scratch responses. A small non-significant negative correlation (−0·0113) was obtained, implying at least that introversion was not a major factor influencing our findings.

These experiments do not answer the question whether the histological and histochemical changes observed in patients with lichen simplex are primary and causative of itching, or secondary to the chronic scratching. The observations of Goldblum & Piper (1954) strongly suggest the latter as the likelier probability, particularly as they were more easily able with their scratching machine to produce patches of lichenification on normal skin, in patients who already had lichen simplex lesion elsewhere. Other changes, such as increased acetylcholine in the skin of patients with atopic dermatitis and neurodermatitis (Scott, 1962), may have some bearing on the itchiness of the skin, leading to further scratching but presumably the histochemical changes occurred after the lesions had developed rather than before. However, the fact that many patients with lichen simplex have an atopic type of skin strongly suggests that ready susceptibility to itching may play a part.

Hence, on the basis of our findings and the histological studies mentioned, one would have to argue that the physical changes are a response to, rather than a cause of the scratching. The fact that patches of lichen simplex occur almost entirely in areas easily accessible to the patients' fingers again speaks in favour of the histology being secondary to repeated trauma. Such changes presumably render the skin more reactive to minor stimuli—as many of our patients had noticed—spontaneous itching being the likely consequence. Examination of itch stimulus thresholds in our patients did not indicate any consistently raised or lowered thresholds, so the patients' own observations are subjective and unconfirmed by our own relatively crude techniques of stimulation.

However, once itching has become established the normal response of scratching becomes a conditioned response, as shown by our findings, with implications for treatment that go beyond the normal practice of antipruritic applications, occlusion and steroid therapy. In short, if a learned response has been established, deconditioning and desensitization may be logical procedures. The likelihood in most cases that spontaneous itching and scratching are responses to emotional tensions should enable the therapist to single out the particular stimuli of an emotional nature that regularly elicit the itch-scratch response, in order, by desensitization, to eliminate it. The recent paper by Bär & Kuypers (1973) certainly demonstrates that behaviour modification in chronic scratchers can be achieved, either by the use of token economy methods or straight-out aversive conditioning. The fact that in some of our patients the lesions had persisted for years, despite appropriate dermatological treatment, suggests a need for psychological assessment and in some instances, specific treatment in conjunction with local applications to obtain more lasting remissions of the disorder.

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The areal extent of dermatophyte infection

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SUMMARY

After stripping with vinyl tape, dermatophytes may be cultured throughout the depth of the stratum corneum. In the present study skin strippings were used to investigate the horizontal spread of the dermatophyte infection.

In five cases of tinea cruris and in twenty-two cases of tinea corporis, fungi could be cultured not only from the visible lesion, but in the majority of cases also from the normal-looking skin up to 6 cm from the margin of the lesion.

No relationship could be found between the size and age of the lesion and the invasion of surrounding, normal-looking skin.

In dermatophyte infections of the skin the entire stratum corneum is invaded by fungal mycelia, as is demonstrable histologically. This vertical extent has been well demonstrated by Marks & Dawber (1972) by the so-called skin-surface biopsy; and by the vinyl tape stripping technique dermatophytes may be cultured from all parts of the stratum corneum (Knudsen, 1971).

In the present study, culture of stratum corneum strippings was employed to investigate the horizontal extent of the fungous infection to decide whether the peripheral demarcation of the infection corresponds to the visible changes, or whether the fungous invasion affects also clinically normal skin.

MATERIAL AND METHOD

The material comprised five cases of tinea cruris and twenty-two cases of tinea corporis (two from the neck, nine from the trunk, and eleven from the limbs).

The duration of the infection had been up to 2 weeks in sixteen cases, 3–4 weeks in three cases, 2–12 months in three cases, more than 1 year in one case, and unknown in four cases.

The mean size of the lesions was 42 × 30 mm in the cases associated with *T. mentagrophytes*, 140 × 110 mm with *T. rubrum*, 123 × 87 mm with *E. floccosum*, and 15 × 11 mm with *M. canis*.

Three cases had already been treated with antimycotics, three with a pure steroid cream, twelve had not had any treatment, and in nine cases no data concerning treatment were available.

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* Read in part at the 20th Meeting of the Scandinavian Dermatological Society, Stockholm, June 1974.

Technical procedure

Using a 1 cm wide vinyl tape (Scotch brand tape 681, Minnesota Mining and Manufacturing Co., Los Angeles, California) the most superficial layer of the stratum corneum was stripped off from the marginal zone of the fungous lesion and radially 1–6 cm into the surrounding, normal-looking skin (Fig. 1). Pieces of these strips 3 cm long were placed, adhesive side down, on the surface of Sabouraud's dextrose agar (with penicillin, streptomycin, and actidione added). The plate was then incubated at 26°C and studied for growth for up to 3 weeks.

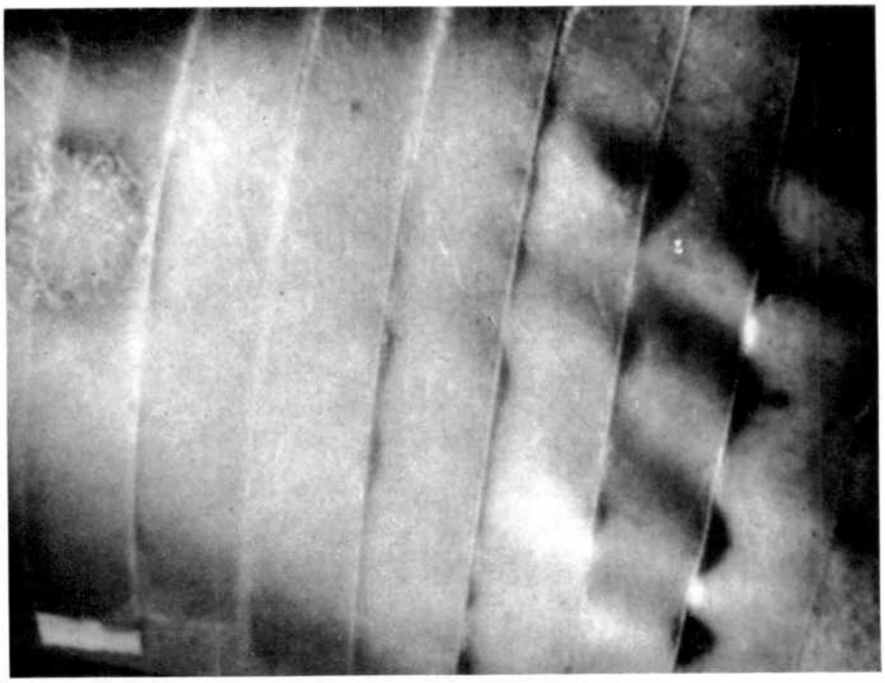

FIGURE 1. Stripping with vinyl tape from fungous lesion and 1–6 cm of the surrounding normal-looking skin.

RESULTS

As is apparent from Table 1, growth of fungi occurred not only from the lesion, but in the majority of cases also from normal-looking skin up to 6 cm from the marginal zone of the lesion.

There was a tendency for growth from the visible fungous infection to appear earlier and with more vigour than from the surrounding skin (Fig. 2).

DISCUSSION

Several authors have been able to isolate dermatophytes from normal-looking skin on the feet. Among patients with asymptomatic ringworm of the feet, Sylvest (1948) found a few having what he called latent infection. English (1969) considered such infections the precursor of symptomatic cases, and according to Marples (1965) dermatophytes may, under ideal conditions, be present as members of the normal cutaneous flora on the feet.

TABLE 1. Numbers of isolations of dermatophytes obtained by vinyl stripping technique from the fungal lesion and at various distances from its border

Species of dermatophyte	No. of cases	Distance from edge of lesion (cm)			
		1	2	4	6
Trichophyton mentagrophytes	5	4	1	2	3
Trichophyton rubrum	4	3	3	2	2
Epidermophyton floccosum	3	3	1	1	1
Microsporum canis	15	12	11	11	10
Total	27	22	16	16	16

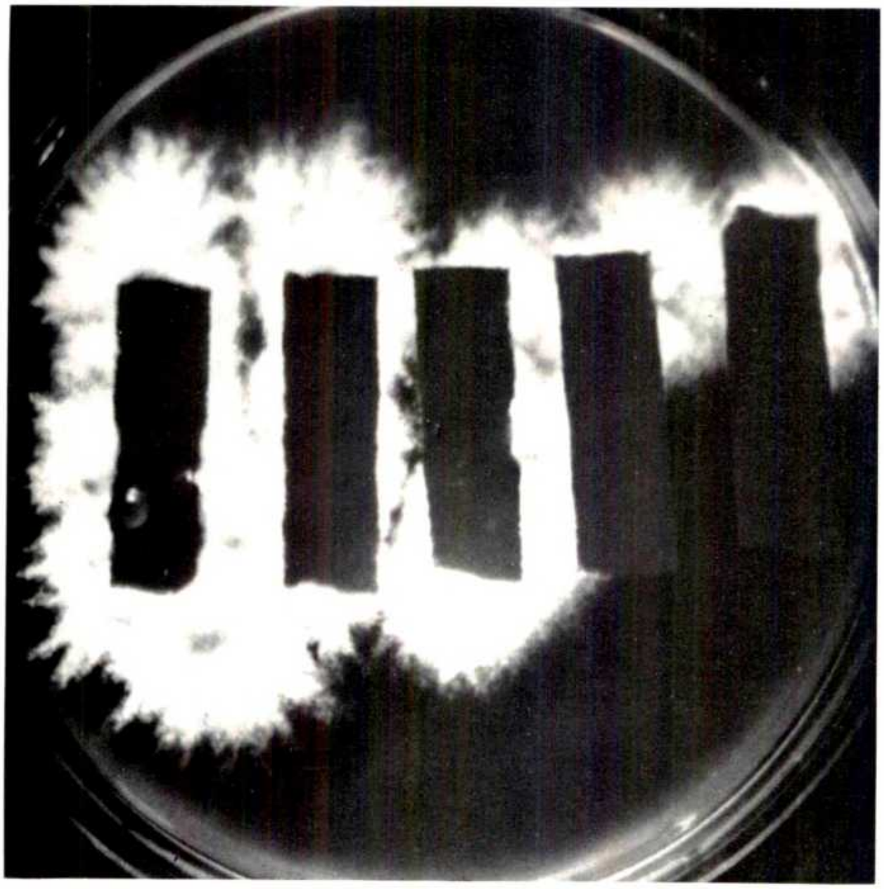

FIGURE 2. Tape culture of *Trichophyton mentagrophytes* on Sabouraud's dextrose agar from the lesion as well as 1, 2, 4 and 6 cm of the surrounding skin.

E

With the exception of palms and soles, asymptomatic infections of the glabrous skin do not occur, in Kligman's (1955) opinion, even in patients with widespread ringworm. However, the present study, which must be considered preliminary, has shown dermatophytes in cultures from normal-looking skin for up to 6 cm from tinea corporis and tinea cruris lesions. Only further investigations can tell how far the latent infection can reach.

New foci of infection may be imagined to arise by spread of spores from a plaque of fungus over the surface of the skin. These preliminary studies indicate, however, that spread around an existing plaque is due to direct outgrowth of mycelia from the fungous plaque throughout the depth of the stratum corneum.

In his excellent study on tinea capitis, Kligman (1952) described a stage of spreading which he compared to the development of a circinate spot on glabrous skin. He found the growth of a spot in the scalp to continue until it reached a diameter of 3–5 cm, and thereafter the hyphae disappeared from the stratum corneum. In the present study it was not possible to demonstrate a corresponding relationship between the size or age of the spot and invasion of the surrounding, normal skin.

According to Beare, Gentles & Mackenzie (1968), the incubation period for a dermatophyte infection is 1–3 weeks. The asymptomatic infection in the periphery of a fungous plaque, as described here, may be interpreted as an infection in the incubation stage.

The practical consequence of the present study must be that in the treatment of ringworm a good portion of the surrounding, normal-looking skin must be included in the field of treatment.

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A study of chemical light screening agents

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SUMMARY

In a group of 104 non-photosensitive subjects and seventy-eight suffering from a variety of photo-dermatoses, the most effective protective preparation against irradiation by short UV sunburn wavelengths (<320 nm) was found to be a mixture of 5% para-aminobenzoic acid and 2·5% of the ester amyl dimethylaminobenzoate in 70% ethanol with para-aminobenzoic acid being usually more effective than the ester, and mexenone the least effective individually. The protective capacity was found to be reduced by showering but not by exercise. The addition of glycerol to the formulations did not affect their protective capacity although the addition of propylene glycol did increase the protection obtained.

In 1971 we published the results of an investigation into the efficacy of certain chemical and physical light screening agents, drawing attention to the importance of relating the action spectrum of the eruption to the absorption spectrum of the agent, and also to the relationship between the results of *in vitro* and *in vivo* assessment (MacLeod & Frain-Bell, 1971). We have reported elsewhere a study of physical screening agents in those photo-dermatoses in which the action spectrum involves long UV (>320 nm) and visible light (>400 nm) (MacLeod & Frain-Bell, 1975). The present report concerns the investigation of three chemical light screening agents—para-aminobenzoic acid (PABA), amyl dimethylaminobenzoate (ester), and mexenone—alone or in combination—to determine which is the most effective and whether a variation in the formulation will alter the protective capacity. In addition, an assessment was made of the significance of the interval between the application of the agent to the skin and subsequent irradiation, and also the effect of prior exercise and bathing.

METHOD

The chemical agents used were PABA (4-aminobenzoic acid; BDH Lab. Reagent), the ester amyl dimethylaminobenzoate (Escalol 506; van Dyk), and mexenone (W.B. Pharmaceuticals).

These agents and various combinations were incorporated into a series of alcoholic solutions as follows:

(A)	PABA	5%	(B)	PABA	5%
	Ethanol	60%		Glycerol	20%
	Water to	100%		Ethanol	60%
				Water to	100%

(C)	PABA	5%	(D)	Mexenone	0·5%
	Propylene glycol	20%		Ethanol to	100%
	Ethanol	60%			
	Water to	100%			
(E)	Mexenone	0·5%	(F)	Mexenone	0·5%
	Glycerol	20%		Propylene Glycol	20%
	Ethanol to	100%		Ethanol to	100%
(G)	Ester	2·5%	(H)	Ester	2·5%
	Ethanol to	100%		Glycerol	20%
				Ethanol to	100%
				(Or commercial preparation in Experiments 2–6)	
(I)	Ester	2·5%	(J)	PABA	5%
	Propylene glycol	20%		Mexenone	0·5%
	Ethanol to	100%		Ethanol to	100%
(K)	Ester	2·5%	(L)	PABA	5%
	Mexenone	0·5%		Ester	2·5%
	Ethanol to	100%		Ethanol to	100%
(M)	PABA	5%	(N)	PABA	5%
	Ester	2·5%		Ethanol	70%
	Mexenone	0·5%		Water to	100%
	Ethanol to	100%			
(O)	Ester	2·5%	(P)	PABA	5%
	Ethanol	70%		Ester	2·5%
	Water to	100%		Ethanol	70%
				Water to	100%
(Q)	PABA	5%	(R)	PABA	5%
	Ester	2·5%		Ester	2·5%
	Mexenone	0·5%		Glycerol	10%
	Ethanol	70%		Ethanol	70%
	Water to	100%		Water to	100%
(S)	PABA	5%			
	Ester	2·5%			
	Mexenone	0·5%			
	Glycerol	10%			
	Ethanol	70%			
	Water to	100%			

For experiments 2–6 the vehicle used in formula H was that of a commercial preparation in which the percentage of the ester (2·5%) was the same but the vehicle differed to some extent although the ethanol content was similar (66%).

Using a Mantoux syringe the preparations were applied in 0·1 ml aliquots to areas of 40 cm^2 of back skin, allowed to dry, then irradiated. The source of irradiation was a xenon arc monochromator system (Mackenzie & Frain-Bell, 1973), the skin of the back being exposed to multiples (usually ×5, 10, 15, 20, and 25, but subsequently modified in the light of experience to ×3, 6, 9, and 12, where necessary) of a previously determined minimal dose for erythema or erythemato-papular response and observed at 7–8 and 24 h intervals, the lower of the two readings being taken as the minimal erythemal dose. The degree of protection, i.e. the protective factor (P.F.), was defined as the mean difference between the dose at which no response was obtained and that required to produce a minimal response. Where the P.F. was greater than the highest multiple dose given, it was taken as the mean between that dose and the next hypothetical dose in the series. This method of statistical evaluation would result in some degree of underrating of the P.F. in some instances.

Experiment 1

In this series formulae A–M were evaluated in a group of forty non-photosensitive subjects, irradiation being carried out at wavelength peak 305 nm ($\frac{1}{2}$ maximum bandwidth, 290–320 nm), the purpose being to compare the relative protective capacity of the chemical agents alone in alcoholic solution (formulae A, D, and G), the effect of addition of glycerol (formulae B, E, and H) or of propylene glycol (formulae C, F, and I) to the vehicle, and to investigate a possible increase in protective capacity as a result of various combinations (formulae J, K, L, and M).

Experiment 2

As the investigation developed it became apparent that the results might be affected by deterioration with the passage of time in the output from the monochromator due to high intensity UV radiation on the surface of the mirrors resulting in a reduction of the shorter UV element and consequent distortion of the waveband pattern; and also to a fall in output due to ageing of the xenon lamp which could not be compensated by increasing the duration of irradiation. In this experiment therefore the three formulations which had been shown to be the most effective in Experiment I (formulae A, H, and M) were evaluated in a group of twenty-four non-photosensitive subjects but this time the irradiation wavelength was narrowed down to 305 nm (bandwidth, 300–310 nm) in an attempt to minimize the effect of any deterioration in output.

Experiment 3

In this experiment a comparison was made using the same formulations A, H, and M, and the same waveband as in Experiment 2 in a group of twelve non-photosensitive subjects, between the protective factor resulting from irradiation immediately after application of the light screening agent and that obtained when irradiation followed (a) exercise (either 5–10 min vigorous exercise (cycling) or 2 h normal activity); and (b) a 5 min warm shower, 2 h after application.

Experiment 4

This was as for Experiment 3 except that the subjects studied consisted of twenty-five individuals suffering from a variety of photodermatoses in whom the action spectrum had already been defined.

Experiment 5

Having evaluated the results from the first four experiments it was considered that the theoretical possibility of deterioration in lamp output affecting the results did not warrant the use of a somewhat artificial narrow waveband, i.e. 300–310 nm which also entailed a relative increase in the irradiation time per subject. For the remainder of the study we reverted to the original broader waveband 305 nm ($\frac{1}{2}$ maximum bandwidth, 290–320 nm) and also the vehicle was standardized to 70% ethanol in water (PABA in Formula N; the ester in formula O; and the combinations of PABA, ester and mexenone in formulae P and Q). The protective capacity of these four formulations was compared with a commercial equivalent (formula H) of the ester in a group of eleven subjects with a variety of photodermatoses and also in fifteen non-photosensitive subjects (Experiment 6).

Experiment 7

In this series the effect on the protective capacity following irradiation at 305 nm (½ maximum bandwidth, 290–320 nm) of the addition of glycerol to the PABA/ester and PABA/ester/mexenone mixture was assessed in a group of twenty-four subjects with a variety of photodermatoses (formulae R and S with added glycerol and formulae P and Q without glycerol) and in a group of 6 non-photosensitive subjects (Experiment 8).

Experiment 9

In this series the protective capacity of the PABA/ester (formula P) and PABA/ester/mexenone (formulae Q) was assessed in a group of eighteen subjects with a variety of photodermatoses when the irradiation at 305 nm (½ maximum bandwidth, 290–320 nm) was carried out immediately after application and compared with that obtained when the irradiation was delayed for 2 h. In Experiment 10 the assessment was the same as in Experiment 9 being carried out in a group of seven non-photosensitive subjects.

RESULTS

The results are shown in Tables 1–10, the table numbers and experiments being the same.

The formulation containing all three active ingredients, PABA, ester and mexenone (formula M), proved to be the most effective with a mean P.F. of 30·9. The PABA formulations A, B with glycerol, and C with propylene glycol, were less effective with P.F.s of 12·4, 11·9, and 19·3 respectively. The combined formulations K (ester + mexenone) and L (PABA + ester) were of similar order with P.F.s of 11·7 and 12·3 respectively. The ester formulations G and H (with glycerol) and I (with propylene glycol) were slightly lower than the PABA formulations at P.F. 9·3, 9·4, and 10·4 respectively, while the mexenone formulations D and E (with glycerol) and F (with propylene glycol) were lower with P.F.s of 3·2, 3·4, and 3·1 respectively. The presence of glycerol (formulae B, E, and H) did not enhance protection but the presence of propylene glycol (formulae C, F, and I) did.

TABLE 1. This shows the protective factor obtained using the formulations A–M in a group of forty non-photosensitive subjects following irradiation at 305 nm peak (½ bandwidth 290–320 nm) carried out immediately after application of the preparation

	A	B	C	D	E	F	G	H	I	J	K	L	M
Mean P.F.	12·4	11·9	19·3	3·2	3·4	3·1	9·3	9·4	10·4	4·8	11·7	12·3	30·9

Table 2 shows the comparison between the protective capacity of PABA (formula A), its ester (formula H) and the mixture of PABA, ester and mexenone (formula M) at the narrow waveband 300–310 nm. The mean P.F.s were 9·8, 6·2, and 8·1 respectively, approximately similar relative findings to the figures obtained after exercise (Table 3: 6·7, 4·3, and 6·7; and Table 4: 9·5, 4·7, and 7·5), showing that PABA alone or in combination with its ester and mexenone is more effective than the ester alone, although overall there was little to choose between the formulations.

The effect of exercise and showering (Tables 3 and 4) is on balance a marginal reduction in protective capacity after exercise and a more significant reduction after showering (Table 3: 3·6, 4·1, and 5·1; Table 4: 4·4, 6·8, and 7·7) with all three formulations.

TABLE 2. This shows the protective factor obtained using the formulations A, H and M in a group of twenty-four non-photosensitive subjects, following irradiation at 305 nm peak (bandwidth 300–310 nm), carried out immediately after application of the preparation

	A	H	M
Mean P.F.	9·8	6·2	8·1

TABLE 3. This shows the protective factor obtained, using the formulations A, H and M in a group of twelve non-photosensitive subjects, following irradiation at 305 nm peak (bandwidth 300–310 nm) carried out (1) immediately after application; (2) following exercise; (3) following showering

	A			H			M		
	Immed.	Exer.	Shower	Immed.	Exer.	Shower	Immed.	Exer.	Shower
Mean P.F.	9·7	6·7	3·6	5·4	4·3	4·1	8·0	6·7	5·1

TABLE 4. This shows the protective factor obtained, using the formulations A, H and M in a group of twenty-five subjects suffering from a variety of photodermatoses, following irradiation at 305 nm peak (bandwidth 300–310 nm) carried out (1) immediately after application; (2) following exercise; (3) following showering

	A			H			M		
	Immed.	Exer.	Shower	Immed.	Exer.	Shower	Immed.	Exer.	Shower
Mean P.F.	8·5	9·5	4·4	7·8	4·7	6·8	9·7	7·5	7·7

The results in Tables 5–10 refer to those obtained after the change in the formulation to the 70% ethanol vehicle and the reversion to the 290–320 nm bandwidth. Since the data shown in these tables contained a relatively larger number of results with a P.F. >20, the statistical analysis was carried out using ranks and not the actual values. In this technique the results for each person were assigned the values 1–5, in such a way that the lowest value was assigned 1 and the highest 5. Where values were equal the average of the appropriate ranks was used.

The test used was the Friedman two-way analysis of variance for ranks. This test produces a χ^2 which has a value of 19·0 with 4 degrees of freedom. This indicates significant differences between the combined and individual preparations at 0·1% level of significance ($P<0{\cdot}001$. $\chi^2=39{\cdot}0$ with 4 degrees of freedom). There was a significant difference between the combined and individual preparations at the 0·1% level of significance ($P<0{\cdot}001$).

These results can be interpreted as showing that the mixtures of PABA plus ester (P) and PABA plus ester plus mexenone (Q) are superior to the others and that the commercially available ester (H) is the least effective with PABA alone (N) holding an intermediate position. The results for the ester (O) are less clear-cut since in the photodermatoses subjects (Table 5) it does less well being similar in effect to the commercially available ester preparations whereas in non-light sensitive subjects (Table 6) it does better than the commercial equivalent and is in fact equal to PABA alone.

TABLE 5. (Eleven subjects with photodermatoses) wavelength 305 nm (½ maximum bandwidth 290–320 nm)

Formulation	Total of ranks
P	31·5
Q	29·5
N	19·5
O	13·0
H	11·5

TABLE 6. (Fifteen subjects who were not light sensitive) wavelength 305 nm (½ maximum bandwidth 290–320 nm)

Formulation	Total of ranks
Q	67·5
P	61·0
N	39·5
O	36·5
H	20·5

The total ranks for the glycerol series are shown in Tables 7 and 8 and these results indicate no significant difference between any of the formulations in both series, suggesting that the addition of the glycerol did not enhance the protective capacity.

Analysis of the results for the PABA/ester (B) and PABA/ester/mexenone (Q) formulations in 70% alcohol irradiated immediately and also 2 h after application are shown in Tables 9 and 10.

This analysis indicated no significant change in protective capacity in either group when irradiation was delayed for 2 h after application.

DISCUSSION

The stage has been reached in the assessment of light screening agents where it is possible to offer guidance as to the most effective agents and also the one most suitable for the individual patient, whether he suffers from one of the photodermatoses or simply experiences an excessive sunburn erythema response due for example to a fair complexion. The disadvantages resulting from an

TABLE 7. (Twenty-four subjects with photodermatoses) wavelength 305 nm (½ maximum bandwidth 290–320 nm)

Formulation	Total of ranks
P	49
R (P + Glycerol)	50
Q	50
S (Q + Glycerol)	41

with $\chi^2 = 1{\cdot}083$

TABLE 8. (Six non-photosensitive subjects) wavelength 305 nm (½ maximum bandwidth 290–320 nm)

Formulation	Total of ranks
P	17·5
R (P + Glycerol)	16·5
Q	12·5
S (Q + Glycerol)	13·5

with $\chi^2 = 1{\cdot}7$

TABLE 9. (Eighteen subjects with photodermatoses) wavelength 305 nm (½ maximum bandwidth 290–320 nm)

Formulation	Total of ranks
P (irradiated immediately after application	42
P (irradiated 2 h after application)	37·5
Q (irradiated immediately after application)	42
Q (irradiated 2 h after application)	38·5

with $\chi^2 = 3{\cdot}827$

TABLE 10. (Seven non-photosensitive subjects) wavelength 305 nm (½ maximum bandwidth 290–320 nm)

Formulation	Total of ranks
P (irradiated immediately after application)	17·5
P (irradiated 2 h after application)	18·5
Q (irradiated immediately after application)	16·5
Q (irradiated 2 h after application)	17·5

with $\chi^2 = 0{\cdot}5$

arbitrary choice of agent without prior knowledge of the responsible action spectrum is recognized and the part that these light screening agents should play in routine therapy is accepted.

In 1969, Pathak, Fitzpatrick & Frenk showed that PABA and its esters were the most effective chemical screening agents available for protection against sunburn UV and later Katz (1970) and also Langner & Kligman (1972) provided evidence favouring a 5% hydro-alcoholic solution of PABA as being the more effective of the two. These latter authors also found that it was necessary for maximum protection to wet the surface thoroughly with the preparation and that repeated daily applications would enhance the degree and duration of protection. Willis & Kligman (1970) found that the amount of protection would vary following swimming but that this would be less if the screening agent was applied a considerable time before the swim; the point however was that the protective capacity of the preparation could be significantly affected by contact with water. They found that the loss of protection was very much less following exercise. Pathak *et al.* (1969) had previously reported results suggesting that bathing and exercise did not affect the protective action of PABA, whereas Katz (1970) did not agree, finding that swimming for 10 min removed the protective capacity although he pointed out that this may have been due to the interval between the application of the PABA lotion and the swimming being 15 min in his study and approximately an hour or longer in other reports. The suggestion is that the greater interval of time is necessary to allow the PABA to become fixed in the skin. We had also noted that the protective capacity of PABA was affected to a variable extent by bathing and exercise and that with the former this was less if the interval between the application to the skin and the bathing was prolonged (MacLeod & Frain-Bell, 1971).

The results of our investigations since 1971 have lead us now to the following conclusions: (1) that PABA and its esters are the most effective light screening agents for use in the protection of the skin against short UV sunburn wavelengths (<320 nm) and that probably PABA is the agent of choice; (2) that a mixture of PABA and the ester is more effective than either one or the other alone; (3) that there would appear to be no significant advantage obtained by the addition of mexenone to the PABA/ester mixture despite the fact that in our earlier studies (see Table 1 formula L, nineteen subjects and formula M, six subjects) we had found that the combination of PABA+Ester+Mexenone had a greater protective capacity than the PABA + Ester mixture alone. At that time the former results were based on the findings in only six subjects and the vehicle was ethanol alone; however the vehicle was subsequently changed and irradiation conditions differed from Table 1 to Table 2. Whether this would still be the case for wavelengths >320 nm remains to be seen although in a limited study of a group of ten subjects with a variety of photodermatoses irradiated at 365 nm peak (bandwidth, 335–395 nm) we did not find that the addition of mexenone to a mixture of PABA plus ester made any difference to the relatively poor protective factor of 3 (MacLeod & Frain-Bell, 1975); (4) that a hydro-alcoholic vehicle containing 70% ethanol is best and that no advantage is to be gained by the addition of glycerol. The addition of propylene glycol however did appear to enhance the protective capacity of the mixture; (5) that a reduction in protective capacity does take place after showering even although this is delayed for 2 h after application suggesting that reapplication is probably indicated after bathing and swimming if a variation in protection is to be avoided. Contrary to our findings in 1971 the reduction in protection following exercise was minimal; (6) although Willis & Kligman (1969) demonstrated that a heightened protection is obtained if PABA is applied 2 h or more before exposure, we failed to confirm this in the case of the mixtures PABA/ester or PABA/ester/mexenone.

These results refer equally as well to normal non light sensitive individuals as to those suffering from a photodermatosis. In the latter in particular however there is sometimes the problem of the irritancy and occasionally also the development of allergic sensitivity. It is for this reason that we did not pursue the inclusion of propylene glycol in the vehicle despite the fact the results suggested that it would increase the protective capacity. We also noted frequently a protective factor of 40 or greater and it may be that the percentage of the chemical agent and/or the alcohol ingredient could be reduced for some individuals with therapeutic gain rather than the reverse.

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The HL-A system and psoriasis

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SUMMARY

A study of 157 psoriatic patients showed that 48·4% were HL-A 17 positive (control 6·0%). The relative risk of psoriasis in HL-A 17 positive individuals (14·7) was significantly higher than that reported from other centres, whilst that for HL-A 13 was lower. HL-A 17 was commoner in female patients and associated with an earlier age of onset of psoriasis than when HL-A 17 was absent. The earlier age of onset in females is largely due to the higher frequency of HL-A 17. Family studies show that often the inheritance of HL-A 17 is a necessary condition for psoriasis to appear and this probably applies in some instances to HL-A 13. It is likely that there is interaction between HL-A 17 and HL-A 13 and other genes predisposing to psoriasis. In some families there appears to be no association between psoriasis and the HL-A system. The general significance of these findings is discussed.

Psoriasis is a disease of complex genetic aetiology in which there is a generalized abnormality of the skin associated with a markedly increased rate of epidermal cell turnover. Previous studies have shown that the HL-A chromosomal region includes a locus or loci which constitute part of the genotype giving rise to a susceptibility to psoriasis (Russell, Schultes & Kubar, 1972; White *et al.*, 1972). This paper describes a study of the HL-A system in another population of psoriatic patients with an analysis of the relationship to sex and age of onset and an investigation by means of family studies of the possible nature of the association.

PATIENTS AND METHODS

One hundred and fifty-seven patients attending a dermatological out-patient department or being treated for psoriasis as in-patients were HL-A typed using a 2-stage lymphocytotoxicity micro-method (Terasaki and McLelland, 1964). In 125 cases typing for twenty-three specificities was carried out and the data from these are used to assess the frequency of the different HL-A phenotypes. In respect of an additional thirty-two patients at the beginning of the study, all the relevant sera were not available but typing for HL-A 17 and HL-A 13 was carried out in the same way. The data from these patients are included in the analysis. In addition to routine assessment of the natural history of the skin lesions an enquiry was made into joint symptoms and an examination of the peripheral and spinal joints was made, appropriate X-ray examination being carried out. A routine enquiry as to the occurrence of psoriasis in first and second degree relatives was made and in selected families each relative was personally interviewed, examined where necessary and HL-A typed. Control blood samples were obtained largely from random blood donors and members of staff, no routine enquiry as to the presence of psoriasis being made. (It is of interest that one control subject, typed as HL-A 17 positive, developed psoriasis for the first time 2 months after testing but was retained in the control series.)

RESULTS

The frequency of the HL-A phenotypes in 125 hospital psoriatic patients is shown in Table 1. It is seen that 50·4% of patients had HL-A 17 compared to 6·0% of controls, the difference being highly significant ($\chi^2 = 94{\cdot}96$, $P < 10^{-9}$). If P is multiplied by the number of specificities tested for, i.e. twenty-three, P is still $< 10^{-9}$. HL-A 13 was found in 7·2% of patients compared to 5·15% of controls (not significantly different).

TABLE 1. Frequency of HL-A phenotypes in patients with psoriasis and controls

Antigen 1st segregant series	Psoriasis (n = 125)	Controls (n = 233)
HL-A 1	61 (48·8%)	82 (35·2%)
HL-A 2	69 (55·2%)	109 (46·8%)
HL-A 3	23 (18·4%)	65 (27·9%)
HL-A 9	14 (11·2%)	45 (19·3%)
HL-A 10	7 (5·6%)	19 (8·2%)
HL-A 11	13 (10·4%)	37 (15·9%)
HL-A 28	3 (2·4%)	12 (6·2%)‡
W 29	15 (12·0%)	16 (6·9%)
W 30, W 31	1 (0·8%)*	12 (6·0%)§
W 32	3 (2·4%)†	12 (5·2%)
2nd segregant series		
HL-A 5	9 (7·2%)	21 (9·0%)
HL-A 7	17 (13·6%)	75 (32·2%)
HL-A 8	21 (16·8%)	74 (31·8%)
HL-A 12	47 (37·6%)	63 (27·0%)
HL-A 13	9 (7·2%)	12 (5·2%)
HL-A 14	9 (7·2%)	17 (7·3%)
HL-A 17	63 (50·4%)	14 (6·0%)
HL-A 27	15 (12·0%)	19 (8·2%)
W 5	7 (5·6%)	32 (13·7%)
W 10	10 (8·0%)	23 (9·9%)
W 15	12 (9·6%)	28 (12·0%)
W 18	2 (1·6%)	10 (4·3%)
W 22	5 (4·0%)	13 (5·6%)

*, 84 tested; †, 123 tested; ‡, 194 tested; §, 199 tested.

In the total series of 157 patients HL-A 17 was found in seventy-six, i.e. 48·4% and HL-A 13 in 15 i.e. 9·6%. χ^2 for the HL-A 17 difference is 94·99 ($P < 10^{-9}$) and for the HL-A 13 difference is 2·823 ($P = 0{\cdot}093$).

In Table 2 a, b the frequencies of HL-A 17 and HL-A 13 respectively in psoriatic patients and con-

TABLE 2. Estimates of relative risks of psoriasis

(a) in HL-A 17 positive versus HL-A 17 negative individuals

Investigation	Psoriasis HL-A 17 +	Psoriasis HL-A 17 −	Control HL-A 17 +	Control HL-A 17 −	Relative incidence (x)	$y = \log_e x$	$w = \frac{1}{v}$	wy	Chi square (χ^2) ordinary	Chi square (χ^2) Woolf (wy^2)
Russell *et al.* (1972)	10	34	8	81	2·978	1·091	3·749	4·09	4·75	4·46
White *et al.* (1972)	40	116	31	355	3·949	1 385	14·556	20·17	30·27	27·93
Seignalet *et al.* (1974)	24	86	9	222	6·884	1·929	5·920	11·42	27·38	22 03
Schunter & Schieferstein (1974)	30	60	38	315	4·145	1·422	12·580	17·89	28·11	25·43
Copenhagen (1974)	29	51	123	1418	6·560	1·881	15·891	29·89	71·52	56·22
Arhus (1974)	21	55	46	516	4·270	1·452	11·176	16·23	26·94	23·57
Present series	76	81	14	219	14·677	2·686	9·851	26·46	94·99	71·07

Overall relative risk = Antilog$_e$Y = 5·35 χ^2 for Y = 215·8 (1 d.f.).
Heterogeneity between centres; χ^2 = 14·87, 6 d.f.; $P<0{\cdot}05$.

(b) in HL-A 13 positive individuals versus HL-A 13 negative individuals

Investigation	Psoriasis HL-A 13 +	Psoriasis HL-A 13 −	Control HL-A 13 +	Control HL-A 13 −	Relative incidence (x)	$y = \log_e x$	$w = \frac{1}{v}$	wy	Chi square χ^2 ordinary	Chi square χ^2 Woolf (wy^2)
Russell *et al.* (1972)	12	32	3	86	10·75	2·375	2·18	5·17	16·81	12·30
White *et al.* (1972)	23	133	19	367	3·34	1·206	9·40	11·34	14·99	13·67
Seignalet *et al.* (1974)	14	96	10	221	3·22	1·170	5·37	6·28	8·03	7·35
Schunter & Schieferstein (1974)	30	60	24	329	6·85	1·925	10·56	20·33	47·17	39·13
Copenhagen (1973)	12	68	65	1476	4·01	1·389	8·76	12·17	19·54	16·90
Present series	15	142	12	221	1·95	0·666	6·19	4·12	2·82	2·75

Overall relative risk = Antilog$_e$Y = 4·052 χ^2 for Y = 83·12 (1 d.f.).
Heterogeneity between centres; χ^2 = 8·98, 5 d.f.; $P>0{\cdot}5$.
v = variance of y.

trols are compared with those found in previous studies. It is seen that the relative incidence (Woolf, 1955) for HL-A 17 is higher and for HL-A 13 is lower than that for the other series. Using Woolf's methods to analyse the composite data (Woolf, 1955), the overall relative incidence of HL-A 17 in psoriasis as compared to controls is 5·535 (χ^2 = 215·84, 1 d.f.). χ^2 for heterogeneity between centres is 14·87, 6 d.f.; $P<0{\cdot}05$. This suggests that there is heterogeneity between the centres. When the data for Liverpool are compared with the combined data from other centres χ^2 = 10·8. The probability of finding a value for χ^2 of this magnitude when comparing any one of the seven sets of data with the rest is less than 0·01.

When the frequency of HL-A 13 is examined in this way, the overall relative incidence of HL-A

13 in psoriatic patients compared to controls is 4·05. χ^2 for heterogeneity between centres = 8·98, 5 d.f., $P > 0·5$, i.e. there is no evidence overall of heterogeneity.

Sex in relation to HL-A 17

There were seventy-one males in the total series of 157 patients and of these twenty-eight had HL-A 17, i.e. 39·4%. The corresponding incidence for the females was forty-eight out of eighty-six, i.e. 55·8%. The females appear to have a higher incidence of HL-A 17 ($P = 0·0412$).

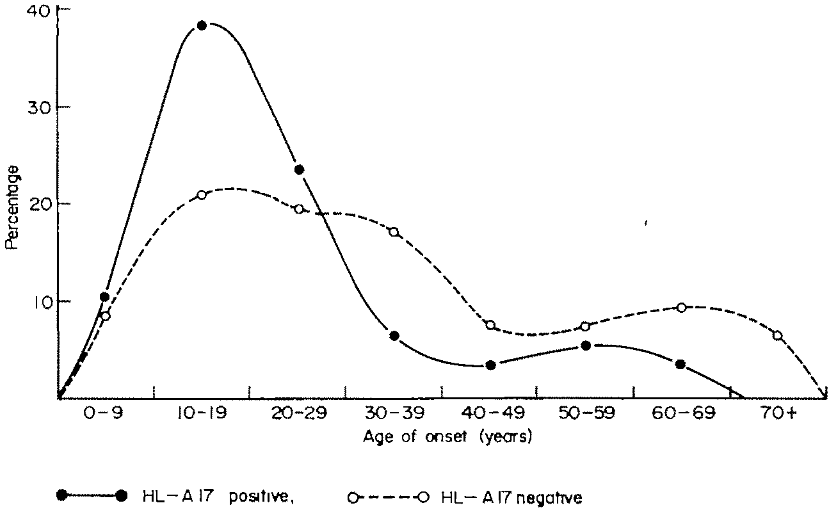

FIGURE 1. Age of onset of psoriasis in seventy-six HL-A 17 positive patients and eighty-one HL-A 17 negative patients, expressed as a percentage.

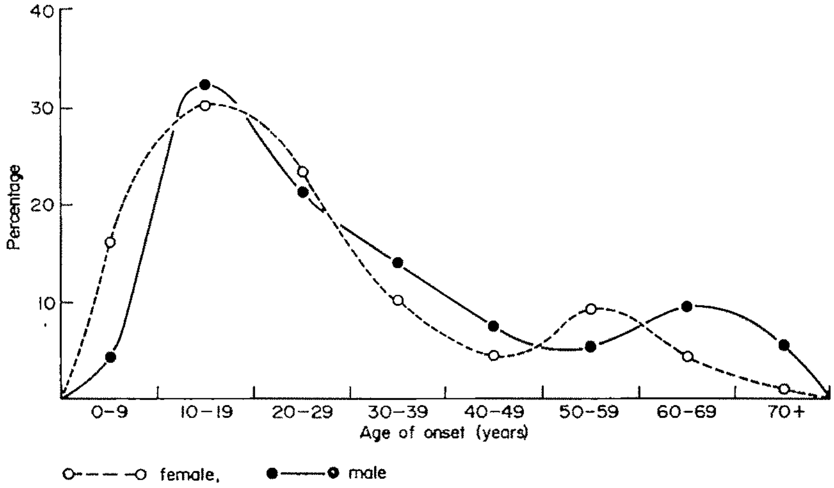

FIGURE 2. Age of onset of psoriasis in seventy-one males and eighty-six females, expressed as a percentage.

Age of onset of psoriasis

The distributions of the age of onset of psoriasis as a percentage of the totals in HL-A 17 positive and HL-A 17 negative patients respectively are shown in Fig. 1. There is a tendency for an earlier age of onset in HL-A 17 positive individuals. Using the Wilcoxon Rank Test the distributions are significantly different ($P = 0{\cdot}008$). Student's *t*-test, to examine the difference between the two mean logarithms of the age of onset, also suggests a difference between the two groups in this respect ($P = 0{\cdot}011$). Fig. 2 shows the age of onset according to sex. There is a suggestion of an earlier age of onset in females but by similar methods of analysis the difference does not reach statistical significance. However, the possibility is raised that the earlier age of onset noted in HL-A 17 positive patients is in some degree due to the excess of females in this group. Therefore the distributions of the age of onset in the HL-A 17 positive and negative patients were compared, taking into account the different proportions of the sexes in the two groups and the data being weighted correspondingly. Using Student's *t*-test there remained a significant difference between the two groups ($t = 2{\cdot}247$; $P = 0{\cdot}025$, 155 d.f.). When males were compared with females, taking into account the different incidences of HL-A 17, no difference in mean age of onset was found.

The conclusion from this is that being HL-A 17 positive *per se* is associated with a tendency to an earlier age of onset, and the observed lower mean age of onset in females than in males is because a higher percentage are HL-A 17 positive.

Presence of arthropathy

Peripheral arthropathy with the features of psoriatic arthritis was noted in thirteen, of whom six were HL-A 17 positive, not significantly different from the other patients. Three were HL-A 27 positive which is suggestively higher than in the control series (23% versus 8·2%). Two patients had sacro-iliitis, spondylitis and peripheral arthritis and were HL-A 27 positive but one with atypical spondylitis without sacro-iliitis was HL-A 27 negative.

Family Studies

In 51·3% of cases where the patient was HL-A 17 positive, a family history of psoriasis was obtained, the corresponding figure for HL-A 17 negative patients being 29·6%. The difference between the two groups is significant ($P = 0{\cdot}0056$). Assuming that the errors inherent in relying on a patient's history of the familial occurrence of psoriasis are the same for both groups, the implication is that there is greater familial aggregation of psoriasis where the proband is HL-A 17 positive.

The chief object of studying certain families was to see whether the inheritance of a particular HL-A phenotype was essential in an individual family for the development of psoriasis. Fig. 3 shows two families, related by marriage. In family A six out of eight siblings in generation II have psoriasis although their parents were apparently unaffected. The commonest haplotype held in common by affected members in this family is 10, w21 (eight individuals). Four other members with psoriasis did not inherit this. Clearly the inheritance of this HL-A phenotype was not essential for the development of psoriasis. If a gene at a locus closely linked to HL-A is postulated as determining the development of psoriasis it would imply that four cross-over events had occurred. It seems likely that in this family several interacting genes are present and there is no evidence that the HL-A system is implicated.

In Family B the proband is HL-A 17 positive and the other members of the family who either have psoriasis or through whom the disease susceptibility appears to have been transmitted (i.e. III, 26 and 27) are also HL-A 17 positive. Other families were studied in which a similar close association between psoriasis and the presence of HL-A 17 was noted, often through three generations.

Fig. 4 shows an interesting family in which the proband is again HL-A 17 positive as are two affected

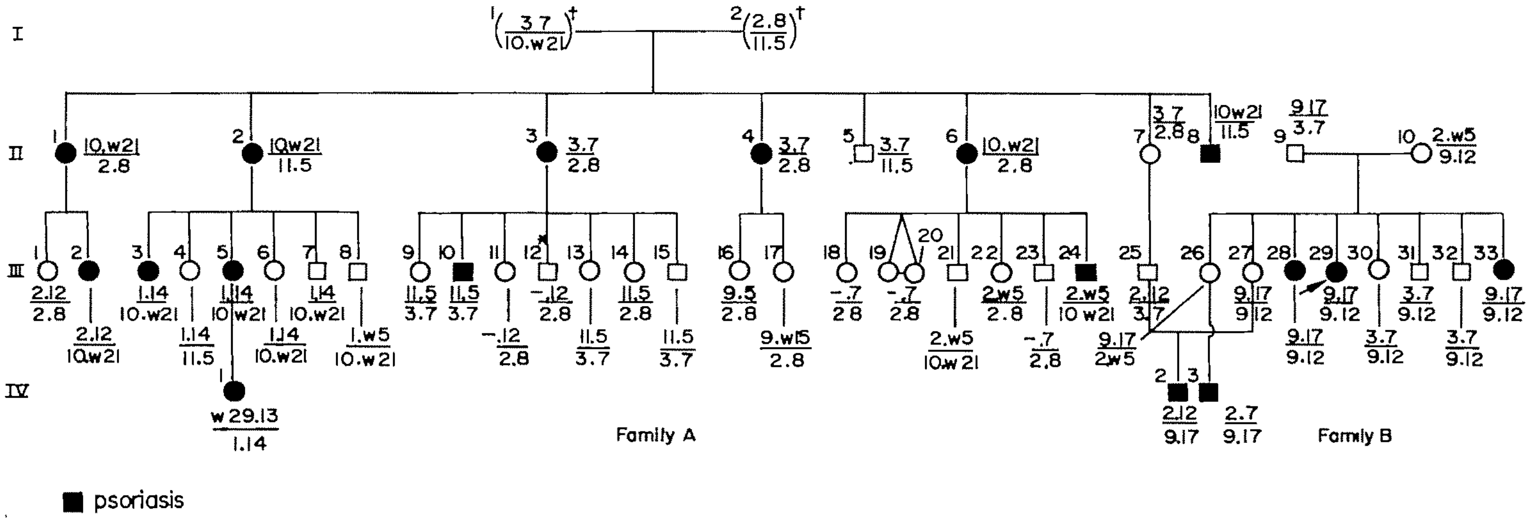

FIGURE 3. HL-A genotypes in the members of the families related by marriage. (– –) refers to deduced genotypes; * (III 12) has had episodic arthritis consistent with psoriatic arthropathy but has no psoriasis.

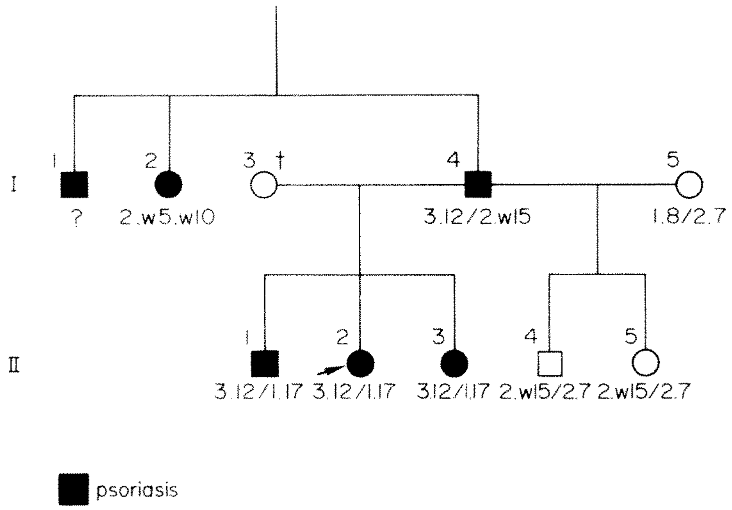

FIGURE 4. HL-A genotypes of a family including six individuals with psoriasis.

siblings. However their father who has psoriasis and spondylitis is HL-A 17 negative as is his affected sister. The HL-A 17 phenotype was inherited from the unaffected mother. Thus other genes predisposing to psoriasis are present. It is of interest that the two somewhat younger children by the second wife (II, 4 and 5) are so far unaffected and it is possible to speculate that the inheritance of HL-A 17 in individuals II, 1, 2 and 3 might have contributed something to the development of the disease.

Examination of all the available data in the families so far studied shows that where the proband was HL-A 17 positive twenty-one of the affected first and second degree relatives have also been HL-A 17 positive and two (see Fig. 4) were HL-A 17 negative. In the families where the proband was HL-A 13 positive five out of six affected first degree relatives also had HL-A 13. (In one family the mother was homozygous HL-A 13 so that all the siblings had HL-A 13.)

In contrast, in families where the proband was HL-A 17 negative, if the haplotype most commonly associated with psoriasis is studied in each family, that particular haplotype was inherited by sixteen affected relatives and was not so inherited by six other affected members. There is a suggestion in this that the development of psoriasis is more closely associated with the inheritance of a particular HL-A phenotype where the proband has HL-A 17 than where he has not.

DISCUSSION

In the original reports of the association between HL-A phenotypes and psoriasis, both HL-A 17 and HL-A 13 were found to be significantly associated with the disease. The results in the population of patients studied in Liverpool show a higher incidence of HL-A 17 and a lower incidence of HL-A 13 than those reported from other centres. It is unlikely that differences in the specificity of the sera used can have played a great part, as typing for these two particular specificities does not give rise to any great difficulty and the control figures in both instances show no significant differences between centres. The explanation is uncertain on present evidence and will ultimately depend on what eventually turns out to be the nature of the association between the HL-A system and the disease. This is further discussed below.

Evidence of an earlier age of onset in HL-A 17 positive psoriatic patients is demonstrated and this was also noted by previous workers (White *et al.*, 1972). It has been repeatedly observed that there is a tendency for female psoriatics to have an earlier age of onset than do male patients (Molin, 1973). In some degree this may be due to a selective bias inherent in studying a hospital population of psoriatics as young females with psoriasis are probably more liable to come to hospital than young males. Independently of this the analysis presented here suggests that the earlier age of onset in females is in large part because of the higher frequency of HL-A 17 in female patients than in males. In this respect it is of interest that Burch & Rowell (1965), in a study of the relative age specific initiation rates of psoriasis in females, were able to distinguish two modes in the distribution of age of onset, the first at about 11 years of age and the second at about 45 years of age. They suggested two genetically distinct sub-populations of females at risk with respect to psoriasis and it is possible that a major component of the genotype in the earlier onset group is in fact HL-A 17.

In most families in which the proband was HL-A 17 positive, the presence of psoriasis in a particular relative appeared to depend on the inheritance of HL-A 17 e.g. Family B in Fig. 3. It thus appears in these families that although other genes predisposing to psoriasis are probably present, HL-A 17 or a closely linked gene is the major genetic factor in producing the disease. In other families HL-A 17 may interact with other genes which themselves can result in psoriasis e.g. the family shown in Fig. 4. The same pattern may apply to HL-A 13.

In general where the proband is HL-A 17 and HL-A 13 negative, a clear association between psoriasis and a particular HL-A phenotype is not usually evident e.g. Family A in Fig. 3. Clearly an interaction of genes unassociated with the HL-A system is likely in such a family and a similar observation was made by Svejgaard *et al.* (1973). Comparing the families in which the proband was HL-A 17 or HL-A 13 positive with those where the proband was negative, there is a suggestion of a greater dependence of the development of psoriasis on the HL-A phenotype in the former than in the latter case. This suggests that HL-A 17 and probably HL-A 13 or a closely linked gene are particularly important genetic factors predisposing to psoriasis. The finding that there is on the whole a greater familial aggregation of psoriasis where the proband is HL-A 17 positive agrees with those of White *et al.* (1972) and Seignalet *et al.* (1974). This also suggests that HL-A 17 or a closely linked gene represents a potent allele in producing psoriasis. The finding in several centres that the HL-A 13 phenotype is also strongly associated with psoriasis in populations suggests that the corresponding gene is also an important genetic factor in these populations.

Family studies suggest considerable genetic heterogeneity in regard to psoriasis (Kimberling & Dobson, 1973). It is likely that genes at several loci affect susceptibility to the disease and in any particular family two or more relevant genes may be interacting. This must be the explanation for the appearance of psoriasis in so many siblings of generation II of Family A (Fig. 3), in which both parents are said to have been free of psoriasis. The HL-A chromosomal region is clearly one at which alleles of considerable effect in producing susceptibility to psoriasis occur in many cases and it is likely that they are usually acting in combination with genes at other loci.

The very considerable studies which have been devoted to the HL-A chromosomal region and to its homologue in the mouse (H-2) and other species point to a very varied and important biological role for the genes in this region (Fogerty International Center Proceedings, 1972). The relevance of this knowledge to studies of human disease susceptibility has been recently reviewed (McDevitt & Bodmer, 1974). The fact that the HL-A genes which are defined serologically occur in a chromosomal region which must include many other loci at which there are genes with important biological effects, including immune response genes, makes it important to consider whether associations of disease with the HL-A system result from the direct involvement of HL-A genes or from closely linked genes. Therefore in considering the ways in which the HL-A association in psoriasis might be operating, two main questions can be posed. Firstly, is the increased susceptibility associated with HL-A 17 and HL-A 13 due to a direct effect of the particular configuration of cell membrane glycoprotein in which the HL-A specificities reside? Alternatively, is the association an indirect one and due to the presence of alleles at a locus closely linked to the second HL-A locus and which themselves predispose to psoriasis? This would imply that such alleles are particularly associated in the population with HL-A 17 and HL-A 13, i.e. a condition of linkage disequilibrium exists. The presence of immune response genes at a locus closely linked to the homologous H-2 locus in the mouse gives theoretical support for this possibility. If the former suggestion were true in most cases of psoriasis, one would expect a strong association with psoriasis in families only for those HL-A phenotypes showing a strong association in the general population of psoriatics. In the family studies so far carried out there is evidence only of HL-A 17 and probably HL-A 13 being major factors in this regard.

If the second suggestion were true it might be expected that if the relevant linked gene is the same, whether linked to HL-A 17, HL-A 13 or to other HL-A genes, then the association between an HL-A phenotype and psoriasis in families should be the same by and large, irrespective of the particular HL-A phenotype involved. The evidence described above is on the whole against this hypothesis because where HL-A 17 or HL-A 13 are not present in a proband the association with a particular HL-A phenotype is less strong than where the proband is HL-A 17 positive. It is however possible to hypothesize that there is in fact a gene of marked effect in producing psoriasis, at a closely linked locus and one which is in linkage disequilibrium particularly with HL-A 17 and HL-A 13. This could explain the observations made in families.

In regard to the differences in the frequencies of HL-A 17 and HL-A 13 in the present study when compared with the data from other centres it is concluded that the relative risk for an HL-A 17 positive individual in the population studied to develop psoriasis is considerably higher than in other populations examined. Conversely the risk for HL-A 13 individuals appears to be lower. It might be that the complex genetic background determining psoriasis in the population studied is different from that in other populations. Also possible is that if there is a particular gene at a locus linked to HL-A and in linkage disequilibrium with HL-A 17 and HL-A 13, the actual degree of disequilibrium might vary markedly from population to population.

The way in which variations in the HL-A phenotype influence susceptibility to psoriasis is a matter for surmise on present evidence. A fundamental change in the skin of psoriatic patients is that the transit time of the affected epidermal cells is shortened (Weinstein & Frost, 1968). A recent study (Orfanos *et al.*, 1973) suggests that lack of contact inhibition at the surface of epidermal cells may be important and that some abnormality in the structure of glycoproteins in the cell membrane might be responsible for this. This raises the possibility that critical changes in the structure of membrane glycoproteins may be influenced by genes in the HL-A chromosomal region.

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Psoriasis in children

A SHORT REVIEW AND A SURVEY OF 245 CASES

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In Scandinavia, psoriasis starts in childhood (before age 16 years) in approximately 45% of cases (Nielsen, 1892; Romanus, 1945; Lomholt, 1963). Denmark alone is estimated to have 35,000 children with psoriasis. A somewhat lower incidence of childhood psoriasis has been found in California (Farber, Bright & Nall, 1968), with 2100 questionnaires showing an onset before the age of 10 in 10% and before the age of 20 years in 25%. In rare cases, psoriasis seems to be congenital, with lesions observed during the first days of life (Lerner & Lerner, 1972). The inheritance pattern remains unclear, but is probably multifactorial (Watson *et al.*, 1972). If one parent has psoriasis, each child has a 25% chance of developing the disease, whereas if both parents are affected, the risk increases to 60–70% for each child (Lomholt, 1963).

Psoriasis is aggravated by psychic stress (Lomholt, 1963; Farber *et al.*, 1968) and infections (Norrlind, 1950; Nørholm-Pedersen, 1952; Asboe-Hansen, 1971; Hellgren, 1964) in at least 45% of cases, while being improved by sun exposure and sea bathing in about 80% (Lomholt, 1963). Children seem especially prone to precipitation of psoriasis by infection, with 69% of initial outbreaks being preceded by infection in one study (Asboe-Hansen, 1971). Guttate lesions in particular often appear explosively after infection, and are frequently very pruritic. These lesions tend to disappear spontaneously within a few weeks to months (Lomholt, 1963), in contrast to longer-lasting but less pruritic guttate lesions of adults.

Several types of psoriasis found in adults are rare in children. These include generalized pustular psoriasis (Crow & Hargreaves, 1968), localized pustular psoriasis of palms and soles, erythroderma (Asboe-Hansen, 1971), and psoriasis with arthritis (Farber *et al.*, 1968; Asboe-Hansen, 1971). Napkin psoriasis affecting the buttocks and perineum (diaper area) is somewhat more common, particularly in young children (Warin & Faulkner, 1961; Andersen & Thomsen, 1971). Nail changes have been found in only 13% of childhood cases (Asboe-Hansen, 1971). Secondary bacterial infection is surprisingly infrequent in psoriasis, despite vigorous scratching, probably because of rapid healing due to high epidermal turnover and almost doubled blood flow (Nyfors & Rothenborg, 1970).

Treatment of psoriasis varies from country to country, but tends to be very costly everywhere

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(Whedon, 1972). Topical creams and ointments alone may cost as much as £2–4 weekly. Ultraviolet light, crude coal tar and other tar preparations, dithranol and topical steroids have all been found to help psoriasis (Knudsen, 1963; Sidi, Zagula-Mally & Hincky, 1968; Farber & Harris, 1970; Watson & Farber, 1971). Grenz rays can also be used safely every 1–2 weeks to a total dose of 1000 rad in any one year (Kopp & Reymann, 1957). Systemic treatment with corticosteroids, methotrexate, or arsenic should not be used in children because of side effects. Prophylactic systemic penicillin may be very helpful if repeated bouts of tonsillitis seem to trigger guttate flares. Tonsillectomy usually is advisable in treatment of recurrent tonsillitis caused by β-haemolytic streptococci (Sidi *et al.*, 1968; Lukovsky *et al.*, 1970). In one study of fifty-seven patients with recurrent tonsillitis and infection-aggravated psoriasis, tonsillectomy produced clearing in twenty-one and improvement in another thirty patients, while failing to help only six (Lukovsky *et al.*, 1970).

A survey of 245 children with psoriasis in Denmark

Case records at the Finsen Institute in Copenhagen contain details of 263 children with psoriasis, of which eighteen had only questionable lesions. Analysis of the 245 positively diagnosed cases using thirty-two parameters (see list) forms the basis of this report.

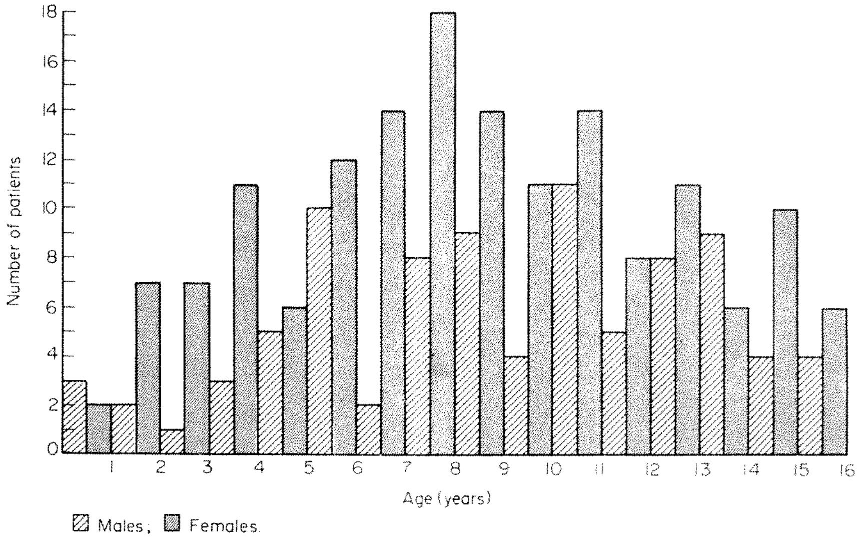

FIGURE 1. Age of onset of psoriasis in 245 children.

RESULTS

Girls greatly outnumbered boys in this study, 64% to 36%. The average age of onset was 8·1 years, being 7·8 years in girls and 8·5 years in boys. The three earliest cases began at 7 days, 14 days, and 6 months of age (Nyfors & Lemholt, 1975), each infant having a strong family history of psoriasis. Fourteen patients in all (6%) developed psoriasis before the age of 2, and another forty-seven (20%) developed it between 2 and 5 years (Table 1). A positive family history of psoriasis was present in 59% of all cases.

Clinical lesions of psoriasis were most frequently guttate, followed next by nummular plaques. Significant itching was mentioned in only ninety cases. The most frequent location of lesions was the scalp (82%), followed by the face (43%). Only 14% had nail changes (Table 2). Psoriasis was continuously present in seventy-one children following the initial outbreak.

TABLE 1. Familial occurrence

	No. of patients	Percentage of total (245)
1. Father	33	13
2. Mother	40	16
3. Siblings	23	10
1+2	3	1.2
1+3	5	2
2+3	9	4
1+2+3	1	0.4
Others	101	41

TABLE 2. Types of lesions and distribution

	No. of patients	Percentage of total (245)
Guttate	108	44
Nummular–guttate	34	14
Nummular	54	22
Plaques	44	18
(Local) Pustular	5	2
Face	105	43
Scalp	202	82
Face + Scalp	102	42
Nails	34	14
Joints	0	0

TABLE 3. Provoking factors after onset

Factor	No. of patients
Sore throat	38
Common cold	10
Sinusitis	1
Chicken-pox	4
Rubella	1
Grenz rays	1
UV and sun light	2

Infection seemed to play a major role in both precipitating and exacerbating psoriasis. Psoriasis began in forty-one patients after clinically recognized infections, particularly sore throats. Exacerbation of psoriasis after tonsillitis occurred in 133 patients, the interval between infection and the outbreak of psoriasis being usually 2–3 weeks. Elevated antistreptococcal titres were found in 147 patients. Sinusitis was found by X-ray in ten patients, although only one patient believed it to be a provoking factor of psoriasis. The common cold seemed to cause exacerbation of psoriasis in ten patients (Table 3).

Other environmental factors which seemed to provoke psoriasis were cold weather and stress. In winter, 81% of the patients had flares of psoriasis, while 90% worsened following stress. In contrast, sunlight and sea bathing improved 75–80% of all cases. A few patients worsened during treatment, due to allergic contact sensitization to medication ingredients. Patch tests showed ten children sensitive to tar, four to mercury sublimate, one to Synalar cream base, one to nickel sulphate, and one to Merbromin.

The various types of topical treatments used are compared in Table 4. Hospitalization on an average of 1·8 times was used in 58% of the children, although 22% had several admissions. The remaining 42% received ambulant treatment only. Various combinations of tar, topical corticosteroids, dithranol, Grenz rays, and ultraviolet light were used in both groups of patients. Tonsillectomy was eventually performed in 19% of the children, with improvement in more than two-thirds (Nyfors, Rasmussen & Lemholt, 1975).

TABLE 4. Treatment and results

Result	Tar	Topical corticosteroid	Dithranol	Grenz rays	UV-light
Improved	161 (66) 94	180 (74) 84	60 (24) 90	161 (67) 90	48 (20) 73
Worse	1 (0) 1	0 (0) 0	0 (0) 0	1 (0) 1	7 (3) 11
No effect	9 (4) 5	34 (14) 16	7 (3) 10	16 (7) 9	11 (5) 16
Not used	70 (29) —	18 (7) —	171 (70) —	58 (23) —	174 (71) —
Not known	4 (2) —	13 (5) —	7 (3) —	9 (4) —	5 (2) —

The first figure indicates number of patients.
The second figure ( ) indicates the percentage of the 245 children.
The third figure indicates the percentage of treated children.

DISCUSSION

The results of this survey agree fairly well with previous studies (Nielsen, 1892; Nardelli, 1957; Lomholt, 1963). In general, children with psoriasis appear to react more easily to physical and psychological trauma than do adults with psoriasis. Children particularly seem to itch more than adults (Lomholt, 1963), and also have greater tendencies to psoriatic exacerbations following infections (Norrlind, 1950; Nørholm-Pedersen, 1952; Lomholt, 1963; Farber *et al.*, 1968; Asboe-Hansen, 1971; Nyfors *et al.*, 1975) and stress (Lomholt, 1963; Farber *et al.*, 1968; Sidi *et al.*, 1968).

Two aspects of treatment deserve special comment. First is the importance of obtaining close parent–child–physician relationships to ensure cooperation with treatment. Ambulatory treatment is definitely preferable and should be tried when possible to eliminate the stress that hospitalization necessarily puts on the child (Bowlby, 1951). A second important aspect of care is the close cooperation between dermatology and otolaryngology. Tonsillectomy to remove foci of infections can be a very valuable tool in the treatment of childhood psoriasis and should probably be encouraged in cases with recurrent tonsillitis.

Unfortunately, record keeping tended to be incomplete in several of the 245 cases studied. As many relevant facts as possible were extracted from the records, working around questionnaire answers such as 'information not available' and 'does not know'. Ideally, special questionnaires should be incorporated into case records to improve their quality for future well-planned prospective examinations of psoriasis in children.

List of parameters studied

(1) age June, 1972
(2) sex
(3) heredity
(4) age of onset
(5) age at first consultation here
(6) onset after infection after . . . weeks
(7) interval between tonsillitis and onset of psoriasis
(8) provoking mechanism
(9) psoriasis free periods
(10) aggravation in relation to—infections (sore throat, cold, sinusitis) ? UV light, grenz rays, children's disease
(11) aggravation in relation to—sunlight
(12) aggravation in relation to—stress
(13) improvement after sea baths
(14) psoriasis worst—summer/winter
(15) tonsillectomy (year)
(16) adenoidectomy (year)
(17) ever treated with—tars, topical corticosteroids, grenz rays, arsenic
(18) nail involvement
(19) psoriatic arthropathy
(20) type of eruption
(21) ESR
(22) haemoglobin
(23) leukocytes
(24) platelets
(25) ASO
(26) ASH
(27) X-ray of sinuses
(28) culture from nose and throat
(29) full-length portrait
(30) positive patch test
(31) latex fixation test
(32) Rose-Waaler test

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The age-of-onset of psoriasis and the relationship to parental psoriasis

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SUMMARY

In a series of 419 consecutive patients with psoriasis the peak age-of-onset was between 5 and 9 years of age in girls and between 15 and 20 years in boys. Subsequently, there was a gradual decline in relation to age.

The incidence of psoriasis in a parent of a proband was shown to be significantly higher in those patients where a detailed examination of the family was undertaken, as compared with the information obtained only from case records.

Psoriasis may manifest itself at any age but in the majority of cases it does so before the age of 30 years. Ingram (1954), in a retrospective review of 1346 patients (58% females), whom he had seen in private practice in the preceding 25 years, noted two peak ages-of-onset, at puberty and at the menopause, and suggested that these could be attributed to hormonal factors. By contrast, Lomholt (1963), who personally examined one-third of the 30,000 inhabitants of the Faroe Islands, found that the median age-of-onset was 13 years in males and 12 years in females. There was a sharp peak age-of-onset between 5 and 9 years in both sexes, this being more marked in females. This difference is important because Burch & Rowell (1965), analysing Ingram's results, postulated that the two peak ages-of-onset signified two genotypes in the population at risk with regard to psoriasis.

There have been several other surveys relating to the age-of-onset. Church (1958) examined 50% of the patients who had attended the Department of Dermatology, Sheffield, 10 years previously; he did not separate the sexes and found a peak age-of-onset between 10 and 15 years with a gradual decrease thereafter. Farber & Carlsen (1966) examined the case records of 1000 patients suffering from psoriasis and showed a peak age-of-onset between 15 and 20 years. Watson *et al.* (1972) sent a questionnaire to over 2000 patients and received replies from 698. The peak age-of-onset in this series was between 10 and 20 years. The sexes were not differentiated in either investigation. Farber & Nall (1974) studied the natural history of psoriasis through a questionnaire survey of 5600 patients throughout the United States; they found that in this series the peak age-of-onset was between 12 and 19 years for females and between 20 and 29 years for males.

In the present investigation it was decided to investigate the age-of-onset of psoriasis in a large series of patients and to determine whether there was any relationship either between the age-of-onset and the clinical pattern of the disease or between the age-of-onset and the presence of psoriasis in a parent of the proband.

METHODS

All patients referred to Huddersfield Royal Infirmary between 1967 and 1973 and diagnosed as suffering from psoriasis were examined personally, great care being taken in determining the age-of-onset. Patients were then divided into two groups. For the first 200 patients seen, a detailed questionnaire and clinical examination were completed with particular reference to the clinical pattern, extent and course of the disease and to the incidence of psoriasis in first degree relatives. When there was any doubt, the relative in question was examined in order to check the accuracy of the information. The second group comprised all patients seen after the first group had been completed. They were examined as carefully as in a routine dermatology clinic, and initial replies regarding the family history were accepted without cross-questioning.

RESULTS

Age-of-onset

Of the 419 patients examined, 241 were female. The ages-of-onset on a 5-year basis are shown for each sex in Fig. 1. This shows that the age-of-onset is earlier in females than in males being between 5 and 9 years and between 15 and 19 years respectively.

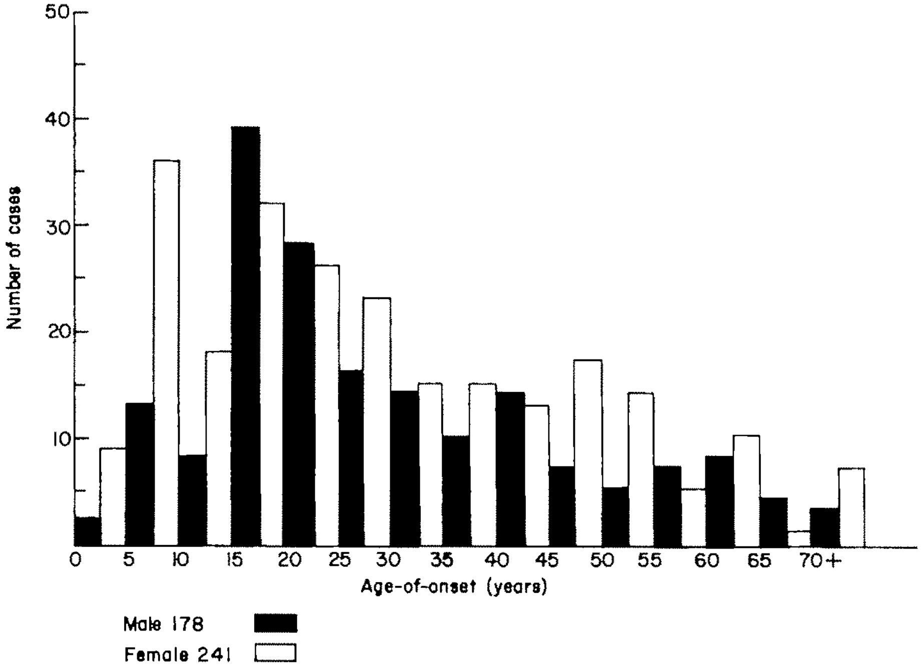

FIGURE 1. Age-of-onset in 419 cases of psoriasis shown at 5-year intervals.

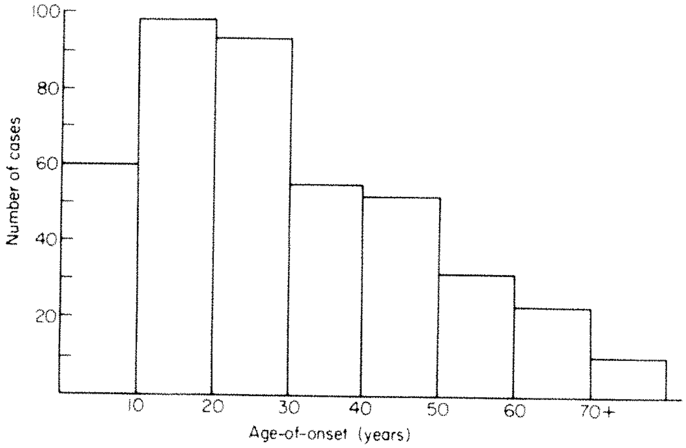

FIGURE 2. Age-of-onset in 419 cases of psoriasis shown at 10-year intervals without sex differentiation.

The results at 10-year intervals, without differentiating the sexes, are presented in Fig. 2 for comparison with the findings of other workers. This shows a peak age-of-onset between 15 and 20 years.

The relative numbers for each decade up to the age of 40 years are shown in Table 1, which shows that by the age of 30 years, 60% of patients had developed psoriasis. In both sexes the frequency of onset fell steadily after the age of 20 years.

Clinical patterns in relation to age of onset

In the first group with 200 patients, for whom the detailed questionnaire was completed, all thirty-one (15·5%) cases of guttate psoriasis occurred before the age of 35 years, and of these twenty-two also had discoid lesions. Of the latter, nineteen progressed to chronic psoriasis whereas only one of the nine cases with pure guttate psoriasis developed discoid lesions during the 5-year period of observation. Otherwise there was no significant correlation between the age of onset and the clinical pattern of the disease.

TABLE 1. Onset of psoriasis in each decade

	Males		Females	
Age-at-onset (years)	No.	(%)	No.	(%)
0–9	15	8·4	45	18·7
10–19	47	26·4	50	20·7
20–29	44	24·7	49	20·3
30–39	24	13·5	30	12·5
40 and over	48	27·0	67	27·8
Total	178		241	
Before age 20	62	34·8	95	39·4
Before 30	106	59·6	144	59·8
30 and over	72	40·5	97	40·2
40 and over	48	27·0	67	27·8

TABLE 2. Incidence of psoriasis in first degree relatives

Patient No.		Proband	1st degree relative	Mother affected	Father affected
1–419	Male 178	44 (10·50%)	51 (12·1%)	15 (3·6%)	10 (2·39%)
	Female 241	79 (18·85%)	92 (21·95%)	26 (6·2%)	23 (5·5%)
1–200	Male 84	29 (14·5%)	32 (16%)	13 (6·5%)	3 (1·5%)
	Female 116	47 (23·5%)	55 (27·5%)	15 (7·5%)	14 (7%)
201–419	Male 94	15 (6·8%)	19 (8·68%)	2 (0·9%)	7 (3·19%)
	Female 125	32 (14·6%)	37 (16·9%)	11 (5·0%)	9 (4·1%)

Incidence of psoriasis in first degree relatives

The incidence of psoriasis in first degree relatives is shown in Table 2 both for the series as a whole and for the two separate groups of patients. It can be seen that the incidence is considerably higher in those patients for whom the questionnaire and detailed examination of relatives were completed than in those examined routinely and that this difference significantly reduces the incidence when both groups are considered together.

Of the 419 patients seventy-four (17·6%) gave a history of psoriasis in a parent, but when this incidence is considered in relation to the first 200 patients only it rises to 22·5%.

Table 3 shows the age-of-onset of psoriasis in those patients in whom a parent was affected.

TABLE 3. Age-of-onset when one parent affected

		Age-of-onset before 15 (years)	Age-of-onset before 20 (years)	Age-of-onset before 30 (years)
Complete series 419 patients	Males 25	5 (20%)	13 (52%)	21 (84%)
	Females 49	18 (36·7%)	31 (63·3%)	42 (85·7%)
Questionnaires completed 200 patients	Males 16	3 (18·75%)	11 (68·75%)	15 (93·75%)
	Females 29	13 (44·8%)	22 (75·86%)	27 (93·1%)
Routine clinic 219 patients	Males 9	2 (22·2%)	2 (22·2%)	6 (66·6%)
	Females 20	5 (25%)	9 (45%)	15 (75%)

For the series as a whole, 85% of patients developed psoriasis before the age of 30 years, this figure rising to 93% when the first 200 patients are considered separately. Both these values are statistically significant but in the second group of 219 patients, the value dropped to 66% and is not statistically significant.

DISCUSSION

The present investigation shows that, when the age-of-onset of psoriasis is considered at 5-year intervals, a peak occurs between 5 and 9 years in girls and between 15 and 19 years in boys. In both sexes there is a gradual decline in incidence in relation to age after these peaks. The findings for girls are in full accord with those of Lomholt (1963), although only 39·4% developed psoriasis before the age of 20 years and 59·8% before the age of 30 years in comparison with 75·5% and 89% respectively in his series.

The peak age-of-onset for boys is 10 years later than Lomholt (1963) observed but only 34·8% developed psoriasis before the age of 20 and 59·6% before 30 years. Corresponding figures in Lomholt's cases were 70% and 92%.

When the sexes are not considered separately and estimations are made on a 10-year basis, the peak age-of-onset is seen to be between 10 and 20 years, which accords with the findings of Church (1958), Farber & Carlsen (1966) and Watson *et al.* (1972). Similarly, the age-of-onset was before the age of 20 in 37% of the patients and before the age of 30 years in 57%. These figures are in close accord with the 35% and 58% respectively found by Farber & Nall (1974) in their questionnaire survey of 5600 patients.

Lomholt's (1963) study is undoubtedly the most comprehensive survey of psoriasis in a population and it would be impracticable to emulate it in this country where the principal source of information is hospital clinics and therefore with biased selection. The present investigation was based on Huddersfield Royal Infirmary which serves a relatively stable population of 225,000. The exact incidence of psoriasis in any population is unknown, but figures ranging from 0·1–2·84% have been given (Farber & Nall, 1974); in this country an incidence of 1–2% is generally quoted. The present series of 419 consecutive cases would therefore suggest a sampling of between one fifth and one tenth of all patients suffering from psoriasis in the area.

In the present enquiry the incidence of psoriasis in first degree relatives is seen to vary according to the method by which the results are obtained. The examination of the first 200 patients was in effect a prospective survey with very detailed confirmation of facts. The information about the second group could be considered as a retrospective survey of case notes and in nearly all instances shows a lower incidence of positive findings. In the first group the incidence of psoriasis in a parent of a proband is 22·5% almost identical with the 23% observed by Lomholt. For the second group of patients this incidence is 14·6%, which is very close to the 14% observed by Watson *et al.* (1972) in their retrospective survey.

In considering the age-of-onset of psoriasis in relation to the presence of the disease in a parent, it is seen that, in the first group 93% of the probands of both sexes had developed psoriasis before the age of 30 years, and that in the second group the incidence was only 66·6% for boys and 75% for girls. The former figure is statistically significant but the latter not. On the evidence of the first group it could be said that, when one or other parent has psoriasis, the disease is likely to be manifest in affected offspring before they reach the age of 30 years.

These differences between the two groups do underline the problems of assessing information from retrospective surveys and of comparing the results of different investigators.

This investigation did not produce any evidence to support the theory of the second genotype in psoriasis as postulated by Burch & Rowell (1965), based on Ingram's retrospective survey. These patients however were all seen in private practice over a period of 25 years by an international authority on psoriasis and as such would form a very highly selected series of cases.

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Seborrhoeic keratoses and carcinoma of the large bowel

THREE CASES EXHIBITING THE SIGN OF LESER-TRÉLAT

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SUMMARY

Three patients who rapidly developed multiple seborrhoeic keratoses and were found to have adenocarcinoma of the large bowel are described. In two cases the discovery of the neoplasm was prompted by skin manifestations.

The small number of cases in which an eruption of seborrhoeic keratoses was a marker of malignancy (sign of Leser-Trélat) have been well reviewed by Dantzig (1973). The validity of the sign has been questioned (Kierland, 1972) because seborrhoeic keratoses and neoplasms are both common in the elderly. We are reporting three cases which controvert this view and emphasize the importance of the clinical sign. Two of the patients were discovered to have neoplasms because they presented to a dermatologist with the rapid development of seborrhoeic keratoses. The third patient developed multiple seborrhoeic keratoses which virtually disappeared following treatment of a primary tumour by radiotherapy, but they recurred when the tumour extended.

Case 1

A 72-year-old man, over a period of 3 months, developed multiple seborrhoeic keratoses over his chest, back and shoulders. They were asymptomatic and he presented only because of their cosmetic unacceptability. On direct questioning the patient admitted that he had alternating constipation and diarrhoea. A mass which was palpable in his epigastrium was found to be a carcinoma of the transverse colon and was resected; there was no evidence of secondary deposits. Three years later, the mass recurred, and a laparatomy revealed extensive carcinomatosis; the patient died 2 months later.

Case 2

A 67-year-old woman developed, over a period of 6 months, multiple seborrhoeic keratoses on her trunk (Fig. 1). Six months later, after she had noticed frank blood on her stools, she was examined under a general anaesthetic. A carcinoma of the rectum, fixed to the sacrum, was found and was thought to be inoperable. Biopsy showed a moderately well differentiated adenocarcinoma; a liver scan revealed no evidence of secondary deposits, and the alkaline phosphatase was normal. She was treated with a course of Megavoltage Radiotherapy, after which her seborrhoeic keratoses virtually cleared and the rectal bleeding regressed considerably. Six months later, however, it was clear that the tumour was extending and the seborrhoeic keratoses were regrowing. Faecal incontinence prompted an abdominal-perineal resection. The histology of the tumour was that of a poorly differentiated adenocarcinoma of the rectum invading perirectal adipose tissue.

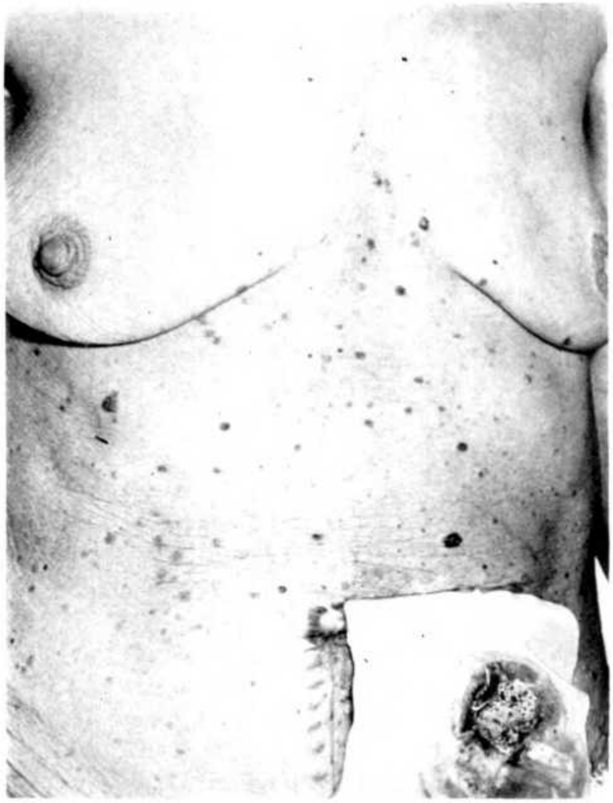

FIGURE 1. Multiple seborrhoeic keratoses of the trunk with carcinoma of the rectum.

Case 3

A 71-year-old man presented with numerous itchy seborrhoeic keratoses situated on his trunk, especially on the back. Direct questioning elicited a history of recent mild weight loss and altered bowel habit, and of one episode of bleeding per rectum. Examination revealed excoriation marks as well as seborrhoeic keratoses on his trunk and more profusely on his back. Haemorrhoids were also found. A barium enema showed a constriction in the proximal arm of the sigmoid colon. At laparotomy an annular carcinoma of the sigmoid loop was resected; it was shown histologically to be highly invasive columnar cell carcinoma. He has remained well but the seborrhoeic keratoses and the pruritus have persisted in the year since the operation.

DISCUSSION

Acceptable case reports of eruptive seborrhoeic keratoses associated with internal malignancy are few and concern the following tumours: adenocarcinoma of the pancreas (Gougerot & Duperrat, 1942), carcinoma of the stomach (Sneddon & Roberts, 1962), carcinoma of the breast (Josserand, 1948), adenocarcinoma of the caecum (Walter, 1972), and Sézary's syndrome (Dantzig, 1973). We agree with the last author that the shoulders are a common site for the seborrhoeic keratoses, but in our cases the keratoses also affected mainly the trunk rather than the extremities. Only one of our cases complained of pruritus and it has been reported in only a minority of the previous cases. The rapidity of the development of the keratoses seems to be a constant feature of all the cases and it occurred in less than 6 months in all our patients.

In cases exhibiting the sign of Leser–Trélat, adenocarcinoma is the commonest type of neoplasm associated with the development of seborrhoeic keratoses and the malignancy is most frequently intra-abdominal. As a marker of internal malignancy the association of acanthosis nigricans and seborrhoeic warts was described in the first documented case by Pollitzer (1890). Since then further cases have been reported (Forman, 1943; Schwartz, 1952; Dingley & Marten, 1957; Ronchese, 1965). None of our cases had acanthosis nigricans, and although Sneddon and Roberts called their cases with seborrhoeic keratoses and internal malignancy an incomplete form of acanthosis nigricans, it may well be that two separate conditions exist.

There are several theories as to why there should be an association of the benign skin change with internal cancer. Curth & Aschner (1959) thought that the skin changes were due to a latent genetic abnormality which is only expressed in the presence of highly malignant carcinoma. Another possibility is that a breakdown in a body defence mechanism allows both the internal cancer and the seborrhoeic keratoses to develop. Seborrhoeic keratoses of the type described here must be distinguished from the acanthomas which develop in areas of erythroderma (Williams, 1956) whether it be due to eczema, to psoriasis, or indeed to an underlying neoplasm. These lesions differ from seborrhoeic keratoses in that they are smaller, only slightly elevated, not nearly so pigmented and the vast majority disappear spontaneously in a few months. The histology of the two lesions is also different.

CONCLUSION

This skin manifestation has been called the sign of Leser-Trélat in recognition of two surgeons, one French—Ulysse Trélat (1828–1890) and the other German—Edmund Leser (1853–1916), whose names are associated with a longstanding belief that an abundance of seborrhoeic keratoses might be a marker of internal malignancy. The origin of the sign in late nineteenth-century foreign literature and the sparsity of case reports make the definition of the syndrome difficult. For practical purposes we suggest that the sudden appearance and the rapid increase in size and number of seborrhoeic keratoses should arouse suspicion of an internal malignancy, most probably of the gastro-intestinal tract.

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M. fortuitum and *M. chelonei*—fast growing mycobacteria

A REVIEW WITH A CASE REPORT

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SUMMARY

Infection due to opportunist mycobacteria is considered in general and that due to the fast growing mycobacteria in particular.

This group is now seen to contain two facultative pathogens, *M. fortuitum* and *M. chelonei* (*abscessus*), all other synonyms for these two species having been shown to be invalid. Both organisms are ubiquitous and normally harmless; however, following injury or injection, they may cause chronic sub-cutaneous abscesses. Pulmonary lesions have also been described. More recently *M. fortuitum* has been found to cause osteomyelitis and corneal ulceration and both organisms have been identified in cases of septicaemia associated with renal dialysis.

In the past *M. chelonei* may have been identified as *M. fortuitum* but although it is more difficult to isolate, it may in fact be the more common cause of disease.

A case is reported of an English girl who received a small injury to her shin in Spain. The initial lesion failed to heal and for 18 months there was repeated formation of small sinuses in the area. Healing then occurred spontaneously. Histology showed a chronic granuloma and on two occasions a rapid growing mycobacteria resistant to all antibiotics was isolated. This was initially identified as *M. fortuitum* but finally as *M. chelonei* and the latter diagnosis was confirmed by differential skin tests. Difficulties in interpretation of these tests are discussed.

The mycobacteria can be divided into three groups. Obligate pathogens such as *M. tuberculosis* and *M. leprae* which do not normally multiply outside vertebrate hosts; facultative pathogens which normally exist as saprophytes in the environment, but sporadically cause disease; and those environmental saprophytes which almost never cause disease.

The facultative pathogens, which include the fast growing mycobacteria, affect animals more often than man. Thus *M. avium*, frequently present in soil, is the exclusive cause of tuberculosis in birds yet is responsible for less than 1% of cases of tuberculosis in man. This group of organisms has come into prominence in the last 20 years, first being known as 'atypical' or 'anonymous' bacteria. They began to be more commonly recognized as a cause of human disease when sensitivity testing against anti-tuberculous drugs again focused attention upon the morphology and cultural characteristics of the tubercle bacillus. Marks & Richards (1962) used the term 'opportunist' to replace the above terms which became obsolete as the individual species of mycobacteria were identified. This term emphasizes that for infection, as distinct from contamination, to occur predisposing factors are generally necessary.

The lungs are the commonest site for these infections (Bates, 1967; Crofton & Douglas, 1969) and there appears to be an increase in their incidence as true tuberculosis dies out (Marks & Jenkins, 1971). This may explain the high incidence of atypical mycobacterial infection found in children with cervical adenitis in Australia (McKellar, Hilton & Masters, 1967) and the U.S.A. (Black & Chapman, 1964). In the United Kingdom, Marsden & Hyde (1962) reviewing ninety-one cases found only one due to *M. tuberculosis* and eleven due to other mycobacteria. A recent survey, however, of a London Borough with a large immigrant population and rising incidence of pulmonary tuberculosis has shown a striking increase also in non-pulmonary disease. Glands are the most common organ to be involved and in all cases the organism has been identified as *M. tuberculosis* either by culture or histology (Mikhail, 1974).

Much more rarely disseminated mycobacterosis occurs, generally in association with immunosuppression. This may be the result of therapy or severe underlying disease and one case has been described with a primary lymphopenic immunological defect (McCracken & Reynolds, 1970). Many of these cases show haematological abnormalities, though whether primary or secondary is hard to determine (*British Medical Journal*, 1968).

Infections of the skin with some of these organisms are familiar to dermatologists, especially the swimming pool and fish tank granulomata caused by *M. marinum* and mycobacterial ulceration in the tropics due to *M. ulcerans*. The abscesses following trauma or injection due to the fast growing mycobacteria under consideration in this paper occur, or perhaps are recognized, much less commonly. Nevertheless, these skin lesions all share certain characteristics, namely, a tendency to chronicity followed by spontaneous resolution, resistance to anti-tuberculous drugs and a granulomatous, or in the case of the ulcerative forms, necrotic histology.

Fast growing mycobacteria

In 1959 Runyon classified the 'atypical' mycobacteria into four groups according to the rate of growth and pigment production of their colonies. In modified form this classification is still used and its most important contribution is the separation of slow-growers (Runyon's groups I, II, & III) from the fast-growers (group IV). These are presently defined by Runyon as organisms able to produce good growth from minimal inocula on egg media at 28°C within 7 days. Like the slow-growers, the fast-growers also include photochromogenic, scotochromogenic and non-pigmented strains or species.

For many years it has been recognized, albeit rarely, that chronic abscesses may be due to non-pigmented fast-growing mycobacteria. These abscesses have either followed injury or injection and the organisms responsible have usually been reported as *M. fortuitum* or *M. abscessus*. With the use of increasingly refined biochemical and serological tests more accurate identification of the individual species of mycobacteria has become possible. For a time it seemed that there was a *M. fortuitum* group comprising several species of which *M. fortuitum* was the major pathogen. Recently, however, it has been shown that there are two distinct organisms responsible for this type of lesion, *M. fortuitum* and *M. abscessus*, more correctly known as *M. chelonei*, of which the latter is probably the commoner cause of disease. These two organisms have both been the source of much nomenclatural and taxonomic confusion.

The earliest name for *M. fortuitum* (da Costa Cruz, 1938) was *M. ranae* (Bergey, 1923), but since this was only recently rediscovered *M. fortuitum* has been conserved by a directive of the Judicial Commission of the International Committee on Systematic Bacteriology (Lessel, 1974) because it is in common use. Other later synonyms for the species which are now invalid include *M. minetti*, *M. giae*, and *M. peregrinum* (Pattyn *et al.*, 1974).

M. chelonei (Bergey, 1923) has one earlier synonym *M. friedmannii* which is invalid since it was not correctly published and several later invalid synonyms *M. abscessus*, *M. borstelense* and *M. runyonii* (Stanford *et al.*, 1972). In their study of forty strains of *M. chelonei* from three continents Stanford *et al.* (1972) showed that these fall into two groups differentiable by biochemical tests and immunodiffusion analysis, but indistinguishable by agglutination tests. These appear to be geographical variants of a single species, one occurring predominantly in Africa and America and the other in Europe.

Thus the fast growing mycobacteria (Runyon's group IV) now comprise the two facultative pathogens *M. fortuitum* and *M. chelonei* as well as a number of saprophytic species.

Because of superficial similarities between *M. fortuitum* and *M. chelonei* insufficient stress is often placed on the necessity for distinguishing between them. However, it is likely that *M. chelonei* is more pathogenic than *M. fortuitum*, the epidemiology of the infection they cause is probably different, and the prognosis of the two infections may also be different.

M. fortuitum

M. fortuitum is a free living organism widely distributed in soil and water. In common with other opportunist mycobacteria it may grow better than *M. tuberculosis* at lower temperatures and it is not pathogenic for guinea-pigs. In the past it was characterized in part by the production of multiple abscesses in mice affecting particularly the kidneys and inner ear. The latter produced a disturbance in balance resulting in so-called 'spinning disease' in which the mice twitch their heads to one side and if held up by their tails spin round (Gorrill, 1956). The organism now called *M. fortuitum* was first isolated from diseased frogs in Germany by Kuster (1905). It was given the name *M. fortuitum* in 1938 by J. da Costa Cruz who isolated a strain from abscesses in the arm of a woman in Brazil following vitamin injections. In 1955 a Maltese girl dug at a carious tooth with a pair of scissors and developed infected submental glands from which *M. fortuitum* was aspirated (Wells, Agius & Smith, 1955). Glandular involvement is however unusual in these infections. In 1960 Brock, Kennedy & Clark described an abscess in the thigh of a woman who had been kicked by a cow; *M. fortuitum* was isolated from the lesion which healed spontaneously after 26 months.

Between 1960 and 1965, 100 patients with injection abscesses due to *M. fortuitum* were seen by Vandepitte, Desmyter & Gatti (1969) in the Congo, and Owen, Smith & Coultras (1963) described an epidemic of fifty injection abscesses in Texas.

From then the organism has been reported, with increasing frequency in American literature, to cause subcutaneous abscesses either following a history of trauma or of injections. Hand & Sandford (1970) gave a useful review of the subject and reported six cases of their own including one with a cauda equina abscess and meningitis. Slotnick & Sacks (1973) reported a case of osteomyelitis and Herndon (1972) three cases of infected wounds obtained in Vietnam. Intractable corneal ulceration following trauma has also been reported (Zimmerman, Turner & McTigue, 1969). Katz & Hull (1971) described septicaemia probably due to this organism as a complication of home dialysis in Texas. The patient used tap water instead of sterile saline to rinse out a piece of apparatus.

M. fortuitum is a much less common cause of pulmonary lesions than other atypical mycobacteria (Phillips & Larkin, 1964). However, lesions clinically similar to pulmonary tuberculosis have been described, usually in older patients and often with pre-existing disease, either tuberculosis or emphysema. Haemoptysis is said to occur frequently. The condition although unresponsive to drug therapy is often benign. Corpe, Smith & Stergus (1961) however described a man with achalasia who developed bilateral cavitating disease due to *M. fortuitum*. He died after 8 years, of cor pulmonale. At post mortem he was also noted to have vertebral osteomyelitis. Far more commonly the organism is grown as a harmless commensal from saliva and sputum, especially in those with chronic obstructive lung disease (Nicholson & Sevier, 1971).

Infection due to *M. fortuitum* has been reported very rarely in England or indeed Europe. Beck (1965) reported three cases of sub-cutaneous abscesses, two following B.C.G. inoculation and one at the site of a previous iron injection.

M. chelonei

M. chelonei is also thought to be ubiquitous but is exceedingly difficult to isolate from natural sources as it is killed by the decontamination methods normally necessary to isolate mycobacteria. It has been cultured from the sides of a cold water tank supplying the pathology laboratory at St Mary's Hospital, Paddington, but has proved difficult to isolate from soil. Natural water is teeming with saprophytic mycobacteria compounding the problem of decontamination. It is also considerably more difficult to isolate from pathogenic material than *M. fortuitum*.

The organism was originally described from diseased turtles in the Berlin Zoo by Friedmann (1903). In 1953, Moore & Frerichs isolated a strain of this organism from the knee and later from gluteal abscesses in a woman from Missouri. It was thought she had had the infection in her knee for 48 years following an injury in a farm yard as a child.

Inman *et al.* (1969) described an outbreak in Hartlepool of chronic recurrent abscesses occurring in twelve patients after histamine injections in the arms. *M. chelonei* was isolated from three of the cases and it was suspected that one of the multidose bottles of histamine solution had become contaminated. Morris & Grant (1972) reported a case of cervical adenitis in a child from which the organism was isolated; this probably followed a dental injection given 4 months previously. In 1973 Borghans & Stanford described a large outbreak of abscesses following diphtheria—pertussis—tetanus—polio vaccination in the Netherlands. Fifty abscesses resulted in forty-seven children and took from 1 to 13 months (mean 3 months) to develop. They pointed out that this delay may mean that the patients did not relate their abscesses to their injections.

In America, Gangadharam & Hsu (1972) described a 17-year-old boy who developed an infection in the thigh after an injury with an iron spike and Gruft & Henning (1972) isolated *M. chelonei* from a pulmonary lesion in an elderly man. In the latter case the organism was isolated only after the concentration of sodium hydroxide used for decontamination was decreased from 4% to 2%. Graybell *et al.* (1974) reported two cases of disseminated mycobacterios is due to this organism in recipients of renal homografts. One of these cases proved fatal after developing osteomyelitis and synovitis necessitating bilateral above-knee amputations. The other developed subcutaneous nodules on the abdomen and limbs, which resolved spontaneously after her steroid therapy was stopped. Both organisms were found to be resistant to all antibiotics.

Some cases previously described as being due to *M. fortuitum* may in fact have been due to *M. chelonei*, particularly in those outbreaks where the source of infection had been hard to identify and where the organism proved difficult to isolate, as in the following case.

CASE REPORT

The patient, an English girl of 21 years on holiday in Spain, received a small cut below her left knee in a motorcycle accident. A single clip was put in at a local hospital without anaesthetic. Ten days later on returning to England the wound was obviously infected. She attended the Casualty

Department at Charing Cross Hospital, London, and was given two courses of antibiotics. Routine swabs, however, were negative for bacterial growth. Initially A.F.B. were not sought. A month later she was referred to the Orthopaedic Department with a small ovoid sinus 1 cm long, extending down to the tibia, which was explored under general anaesthesia 3 weeks later. The base was curetted and the edge excised and a secondary suture was performed.

At this point the laboratory reported the isolation on two occasions of a rapidly growing mycobacterium. This was later shown to be resistant to all anti-tuberculous drugs and was preliminarily identified as *M. fortuitum*. During the next 6 months the wound remained apparently quiescent but retained a dry scab and was slightly tender. She then developed an area of erythema and induration at the side of the scab and another small sinus formed. She was referred to the Skin Department and a biopsy showed chronic granulomatous tissue with areas of necrosis but no caseation, compatible with tuberculosis. Her Mantoux 1:1000 was very strongly positive (she had had BCG at the age of 13 years). X-rays of chest and leg were normal and her ESR was 3 mm/h. Treatment was begun with dapsone 100 mg daily but after a further 3 months a fresh sinus appeared and discharged and the dapsone was stopped. Further Lowenstein cultures were negative and no other organisms were grown. Over the next 6 months two more small sinuses appeared and discharged and it was decided that excision of the whole area was necessary. However, at this point, 19 months after the injury, the lesion settled down and at 2 years there was a well-healed scar which has remained healed. Differential skin-testing was then done using extracts prepared from sonically disrupted bacilli, with three immunodiffusion serotypes of *M. fortuitum* (Ranin I, Ranin II and Ranin IV), one strain of *M. chelonei* (Chelonin) and 1:10,000 old tuberculin. The patient was strongly positive to Chelonin and positive to the old tuberculin and Ranin II and IV (see Table 1).

TABLE 1. Results of intradermal skin tests

Reagent	Dose	Result Diameter of induration (mm)
O.T.	1 in 10,000	9
Chelonin	100 units	19
Ranin I	100 units	—
Ranin II	100 units	5
Ranin IV	100 units	12

Since previous reports have shown that infection with *M. chelonei* does not produce a positive tuberculin reaction, we assume that our patient's positive reaction was due to her previous BCG. However the reactions to the Ranin preparations probably represent cross reactivity with *M. chelonei*.

More recently the organism originally cultured from this abscess has been re-examined with cultural, biochemical and serological tests designed to differentiate *M. fortuitum* from *M. chelonei* and has been shown to be *M. chelonei*. This probably accounts for the difficulty encountered in obtaining further cultures and is substantiated by the strongly positive skin test.

Skin tests in mycobacterial infection

The interpretation of skin tests in these conditions is complicated by cross-sensitization due to shared antigenic structures. In the past a positive tuberculin test was thought to indicate infection with *M. tuberculosis*. However, when the results of world-wide skin testing prior to BCG vaccination were examinated it became clear that whilst this was true for a strong reaction, weak reactions often occurred unrelated to tuberculous infection (Edwards & Palmer, 1953). The true incidence of sub-clinical infection with opportunist mycobacteria is still far from clear due to difficulty in interpretation of results.

Edwards (1963) reported the results of differential skin tests in a large number of recruits to the U.S. Navy. A surprisingly high overall reactor rate was found with marked geographical variation. It is interesting that all the antigens apart from *M. fortuitum* gave a higher percentage of positive results than *M. tuberculosis*. Smith (1967) surveyed the quantitative significance of tuberculin tests and considered the influence of the atypical mycobacteria upon them.

In the British Isles the British Tuberculosis Association (1965) concluded that in rural areas of England and Wales weak reactions to human tuberculin were in fact mostly due to cross reaction with *M. avium*. Keay & Edmunds (1966) using several different PPD's showed a widespread prevalence of sensitization to atypical mycobacteria.

The presence of multiple positives in one person may indicate cross reaction in which the largest reaction is usually due to the pathogen or may be due to multiple sub-clinical infections. The use of skin test reagents prepared from ultrasonic extracts of mycobacteria, such as the Chelonin and Ranin used in this study, may well prove much more specific than either old tuberculin or PPD. Further work using these may yield valuable information as to the true incidence and significance of sub-clinical infection with these organisms.

ACKNOWLEDGMENTS

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Case Reports

Wegener's granulomatosis—controlled by azathioprine and corticosteroids

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SUMMARY

A case of Wegener's granulomatosis is presented with control of lung lesions by corticosteroids and renal lesions by azathioprine. It supports previous experience of antimitotic therapy in this condition.

Wegener's granulomatosis is a rare disease characterized by necrotizing granulomatous involvement of the upper and lower respiratory tract. There is a generalized focal necrotizing vasculitis involving both arteries and veins, almost always in the lungs and more or less widely disseminated in other sites, also focal or diffuse glomerulitis (Godman & Churg, 1954). Clinically it is manifested by intractable sinusitis, bloody nasal discharge, cough, dyspnoea, chest pain, buccal and palatal ulceration, papulonecrotic skin lesions on extensor surfaces, and renal failure.

Midline lethal granuloma is a locally destructive lesion of the nose which may lead to death from haemorrhage and systemic toxicity. In one form, it represents a localized form of Wegener's granulomatosis, but may become systematized with vasculitis. However, it may remain localized to the nose or a similar picture may be caused by reticulum cell sarcoma.

Untreated, the average life span in Wegener's granulomatosis is 5 months (Aldo *et al.*, 1970) but in the early nineteen sixties, systemic steroids improved this situation. The advent of antimitotic therapy in the late nineteen sixties offered for the first time the chance of prolonged survival (Aldo *et al.*, 1970; Editorial, 1972).

CASE REPORT

The patient, a 49-year-old housewife, was admitted in July 1972 to an orthopaedic ward with a rocky hard mass in the left upper outer arm. It had been present for 3 months. In the ward, she was noticed to be an ill, anaemic woman who had a hoarse voice and signs of consolidation in the right lung. To eliminate the possibility of the mass being a secondary deposit, a biopsy was performed. The report was, 'The epidermis shows hyperkeratosis and acanthosis. There is a perivascular infiltrate of neutrophils, eosinophils, lymphocytes and histiocytes in the upper dermis. The lower dermis contains several tuberculoid granulomata including multinucleated giant cells and the subcutanea shows panniculitis' (Fig. 1). Her Hb was 55%, ESR 110 mm/h and total white count 12,000/mm^3 with a normal differential (71% polymorphs). The urine contained albumin and there was a coliform infection.

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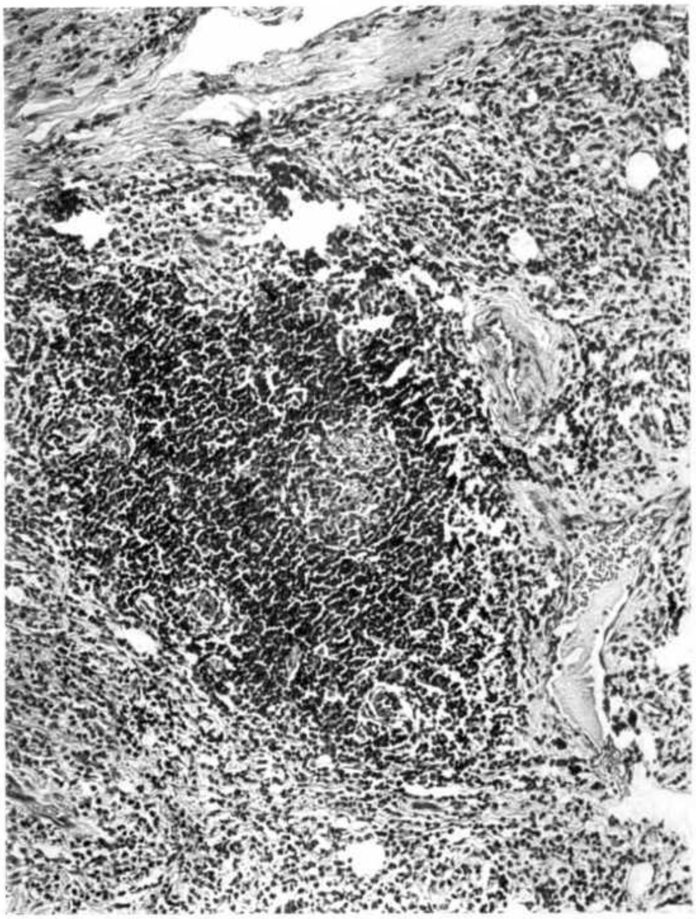

FIGURE 1. Deep dermal granuloma (H & E, × 125).

An X-ray of the chest was then reported as, 'There are two irregular thick walled cavities with fluid levels in the right lung—one in the upper lobe and one in the apical segment of the lower lobe' (Fig. 2).

Her condition deteriorated further with cough, anorexia and the development of papulo-necrotic lesions on the dorsa of the hands, wrists, extensor aspects of the forearms, elbows, back, trunk and legs. She was noticed to have necrotic ulcers on the tongue and buccal mucous membrane and she could now speak only in a whisper.

In a moribund state, diagnosed clinically as Wegener's granulomatosis, she was transferred to the dermatological ward where she was transfused with 3 pints of blood and commenced on prednisolone 60 mg daily. Her improvement was dramatic.

Mr A.G.Norman performed a bronchial biopsy at this point which was reported as 'An area of consolidation is seen within the lung parenchyma. Surrounding an artery showing gross changes of an arteritis involving the intima, is an area of fibrinoid necrosis and a dense inflammatory infiltrate containing plasma cells and neutrophils.'

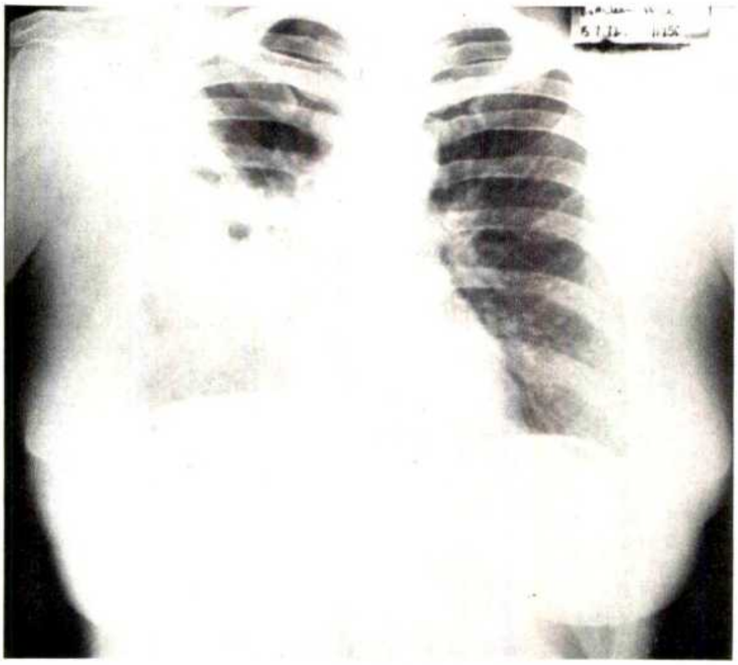

FIGURE 2. Chest X-ray.

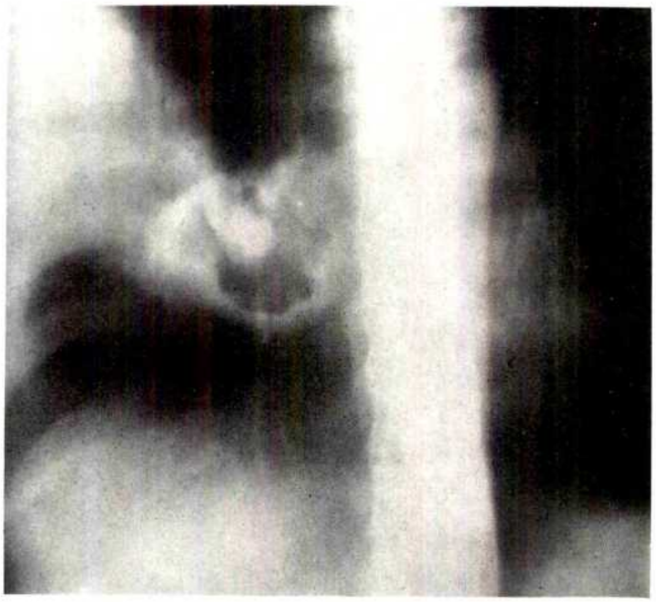

FIGURE 3. Tomography showing cavitated lesion.

Tomography of the right lung confirmed the appearance of irregular nodules of tissue contained within cavities. The appearances were consistent with Wegener's granulomata (Fig. 3).

Shortly after this, she developed a left sided palmar arch thrombosis with gangrene of the tips of the ring and little fingers.

By this time, her well-being was greatly improved and her skin and mouth had healed. Further investigation of renal function showed her to be excreting 0·65 g of protein a day in the urine and her creatinine clearance was reduced at 60 ml/min.

Normal investigations included serum proteins and electrophoresis, blood urea, blood sugar, serum and 24 h urinary electrolytes, serum calcium, phosphates, uric acid, bilirubin, immunoglobulins, coagulation profile, E.C.G. and I.V.P. The sputum was sterile and contained no neoplastic cells. Antinuclear factor was negative and no mitochondrial or smooth muscle antibodies were detected. Immunofluorescent studies of a renal biopsy showed no immunoglobulin or complement fixation in glomeruli, tubules or interstitial tissue.

As the patient was now being considered for azathioprine therapy, Miss M. Platts was asked to perform a renal biopsy. This was reported as, 'Some glomeruli are normal but others show areas of focal fibrinoid necrosis with capsular adhesions and occasional crescent formation. The blood vessels are normal and no arteritis is present. Tubules are also normal but the interstitial tissue shows a chronic inflammatory infiltrate. The appearance is that of Wegener's granulomatosis or microscopic polyarteritis nodosa' (Fig. 4).

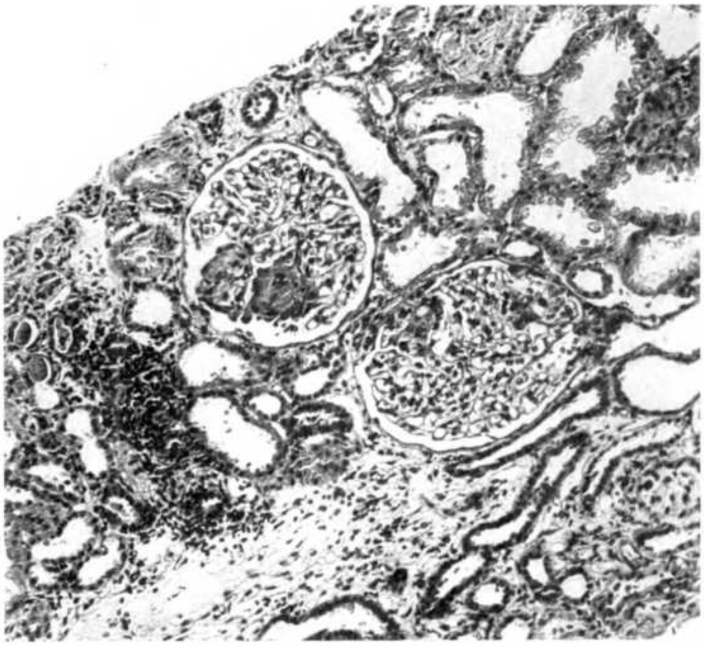

FIGURE 4. Renal biopsy showing glomerulitis (H & E, × 125).

She was started on azathioprine in early August with the news that the urine contained granular casts, epithelial cells, 40 white blood cells and 20 red cells in each high power field and 24 h urinary protein had risen to 5·4 g. By discharge this figure had fallen to 0·8 g daily.

When she was readmitted in early September, her Hb was 91%, ESR 11, urine sterile and 24 h urinary protein was negligible. Her blood urea, however, was raised at 58 mg/100 ml and has remained at around this level since. Her B.P. was 170/110.

Prednisolone was reduced to 20 mg daily. At the end of October, she complained of headaches, diplopia, hoarseness and dysphagia. She was also ataxic. Admitted to hospital, she had signs of a right sided brain stem infarction. With tube feeding she improved. Prednisolone was increased to 30 mg daily and azathioprine to 150 mg daily. An E.N.T. opinion was that the nose was not involved by granulomatosis. Neurological recovery was virtually complete by discharge in November, 1972.

In January 1973, the chest X-ray was clear apart from residual shadowing in the right upper lobe. August saw her complaining of thoracic spine pain and this became so bad that, by October, she was confined to a wheel chair. She was admitted to hospital and found to have compression fractures of D.12 and L.3 vertebral bodies on X-ray. There was generalized osteoporosis. Her prednisolone was reduced, she was placed on an 800 calorie diet and a lumbar brace was fitted. On this regime, her Cushingoid appearance improved, her back pain was relieved, her blood pressure settled and her blood sugar fell to normal. Azathioprine was increased to 200 mg daily and prednisolone decreased to 10 mg daily. The 24 h urinary protein was 1 g and creatinine clearance 46 ml/min.

Currently (April 1974) she is well on 200 mg azathioprine and 5 mg prednisolone daily. Regular measurements of white cell and platelet count over the period of therapy have shown no depression.

Immunology

The cause of Wegener's granulomatosis is unknown but an immunological one is suspected. Few studies have been made of immunological function in the disease. Kaplan, Hayslett & Calabresi (1968) showed not only arrest of renal and improvement of pulmonary disease in two patients treated with azathioprine but also suppression of both humoral antibody levels and delayed type hypersensitivity to a new antigen. Pre-existing immunity was not affected. Fauci, Wolff & Johnson (1971) reported nine patients. Untreated the patients had raised levels of IgA. Their findings on treatment with cyclophosphamide broadly supported the above report. Seven of the nine patients had undetectable humoral and delayed hypersensitivity responses to a new antigenic stimulus but five of the seven retained previously established delayed hypersensitivity. A favourable clinical response to cyclophosphamide and immunosuppression appeared to be related. Shillitoe *et al.* (1974a) studied ten patients with either Wegener's granulomatosis or midline lethal granuloma and also found IgA levels raised. Further, there was impaired skin delayed hypersensitivity in eight and impaired lymphocyte transformation in six. However, there was intact macrophage migration inhibition and they concluded that the cell mediated immunity defect was partial as in chronic mucocutaneous candidiasis and sarcoidosis. Since two untreated patients had the same findings they speculated that partial immune deficiency may be involved in the pathogenesis of the disease. This view was challenged by Fauci & Wolff (1974) who said that, in their studies of a further eleven patients, some on and some off treatment with cyclophosphamide, delayed hypersensitivity was intact. They contended that immunosuppressive treatment, especially with corticosteroids, may have caused the change in immunological function. In reply Shillitoe *et al.* (1974b) said that many of their patients had active disease when tested, while those of Fauci & Wolff (1974) were in remission. They emphasized that skin tests alone do not give a complete assessment of cell mediated immunity.

Treatment

Fahey *et al.* (1954) reported a short course of nitrogen mustard in one case. Bouroncle, Smith & Cuppage (1967) reported the first case of successful azathioprine treatment in Wegener's granulomatosis. It was the only agent used and X-rays of the chest and renal biopsies both improved. In 1969, Peermohamed and Shafar reported not only the control of Wegener's granulomatosis by azathioprine but also continuation of remission after treatment was stopped.

Evidence of the dramatic effect of antimitotic agents in treatment was provided by Aldo *et al.* (1970). In their review of fifteen cases the average life span (5 months) and 1 year survival rate (18%) of the untreated cases was compared with 12½ months and 34% on steroids and with over 18 months and 80% on antimitotic agents. This was supported in an editorial (1972) in which forty-six cases were reviewed. All except four had survived at least 1 year. Both alkylating agents (including nitrogen mustard, cyclophosphamide and chlorambucil) and antimetabolites (including azathioprine, 5-fluorouracil and methotrexate) have been used. Although Raitt (1971) said there was no advantage in any particular antimitotic, Kaplan & Calabresi (1973) preferred azathioprine as it has a low incidence of side effects.

The mechanism by which these agents exert their action appears uncertain at present. Possible mechanisms suggested include an anti-inflammatory action (although alkylating agents which are effective in the disease do not possess much of this activity) and immunosuppression. Paradoxically, steroids are potent immunosuppressives but are not as effective as antimitotics in controlling the disease. It may be that a cytotoxic action is involved which is analogous to the beneficial effect of radiotherapy in lethal midline granuloma.

Trethowan, McEvedy & King (1973) reported a case in which radiotherapy to ischaemic fingers led to healing so that amputation was avoided. They thought it likely that the localized vasculitis was suppressed. This again is similar to the effect of radiotherapy in lethal midline granuloma.

DISCUSSION

The patient in this case report had typical Wegener's granulomatosis and was dangerously ill at the time of presentation. Since the activity of azathioprine is delayed, the speed of steroid action was valuable in reviving this moribund patient. Her response to steroids was impressive and immediate but her renal disease deteriorated, as in a case of Hollander & Manning (1967). She also developed steroid induced diabetes and a Cushingoid appearance. With the use of azathioprine there was a gradual return of renal function towards normal. The episode of brain stem infarction probably occurred because of insufficient control of the arteritic process with steroid reduction and responded to an increase in both steroids and azathioprine. The final disabling complication of osteoporotic spine collapse was controlled by reduction of steroids while the total disease was suppressed by a further increase in azathioprine.

In this case immediate resuscitation was secured by steroids. It emphasized that Wegener's granulomatosis may be controlled in the long term by antimetabolite therapy virtually alone. It suppresses the disease more satisfactorily than do steroids and with fewer side effects. It supports previous reports of the efficacy of antimitotic agents.

Postscript

In October 1974 the patient's corticosteroids were stopped. Within a fortnight she developed a cough, and chest X-ray showed further right sided lesions. Prednisolone 30 mg daily was started with some improvement. However, her renal function tests, including serum creatinine, remained normal, and 24 h urinary protein was negligible. Thus it appears that the small dose of prednisolone was controlling the lung disease and that azathioprine alone was capable of suppressing only the renal disease.

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Anonychia with bizarre flexural pigmentation—an autosomal dominant dermatosis

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SUMMARY

A nulliparous female aged 26 years, who presented with long-standing sore and cracked soles, showed congenital anonychia, long-standing hyper- and hypo-pigmentation in axillae and groins, dry palmar and plantar skin as well as other skin and hair abnormalities. She gave a family history of nail, hair and pigmentary abnormalities and examination of living affected relatives confirmed this.

This appears to be a hitherto undescribed autosomal dominant syndrome.

CASE REPORT

Miss P.R. (III.3—see Fig. 1) aged 26 years, attended with the complaint of recurrent soreness and cracking of the plantar skin since the age of 6 months; her palms were similarly affected but to a lesser extent and only over recent years. Finger and toe nails had been absent from birth. Although not mentioning sweating, on direct questioning she claimed to sweat very little. Her mother (II.5), a deceased maternal uncle (II.4) and a younger brother (III.4) were said to have always had dry palmar skin, absent nails and slowly-growing coarse scalp hair. Her deceased maternal grandmother (I.2) was stated to have been unaffected but details of the maternal grandfather (I.1) were not known. There was no family history of cousin marriages and neither a personal nor a family history of atopy.

On examination, she showed dry thinned peeling palmar and plantar skin, and discrete superficial red punctate clefts were visible over the palms and lateral border of the insteps. The main flexion creases over the hands were present in addition to increased palmar markings but epidermal ridges over fingertips and palms were not clearly visible (Fig. 2). However, some ridges over her hands (and feet) could be seen with magnification but, because of the skin state, fingertip patterns were too distorted to be decipherable. Localized hyperkeratosis was present over the distal soles and plantar aspect of the heels.

Finger nails (Fig. 3) and most toe nails were absent but poorly developed toe nails were present on the 1st and 3rd left toes and 1st and 2nd right toes.

Faint discrete small macular telangiectases were visible over the front of the knees and a minor degree of follicular hyperkeratosis was present over the limbs.

The skin of the axillae (Fig. 4), groins and natal cleft presented an odd mixture of hypo- and hyperpigmentation but hairs in these areas were pigmented normally and the skin was not atrophic. There

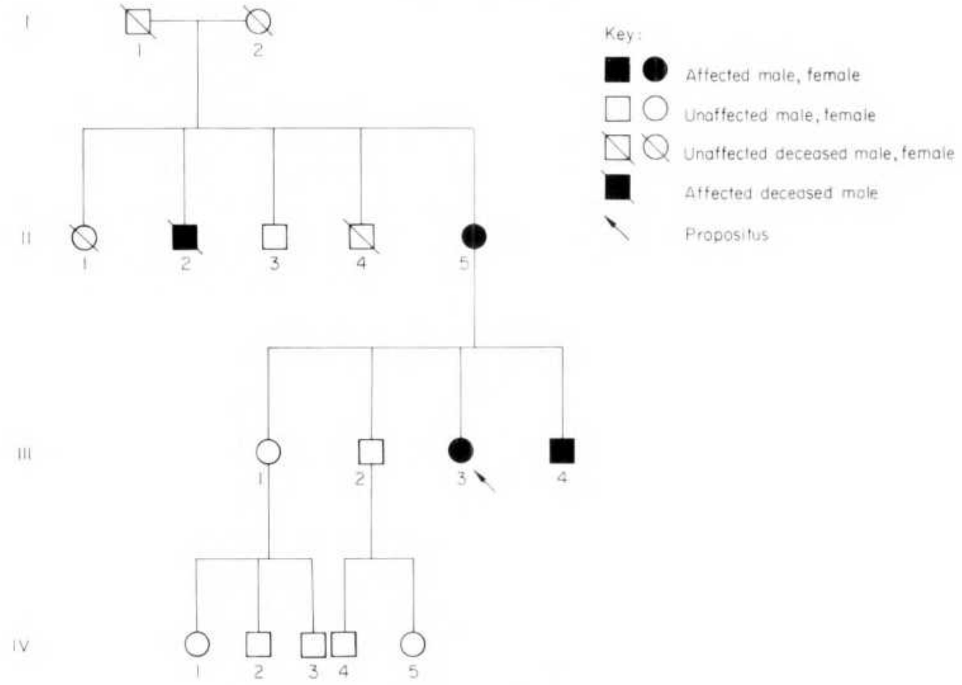

FIGURE 1. Pedigree.

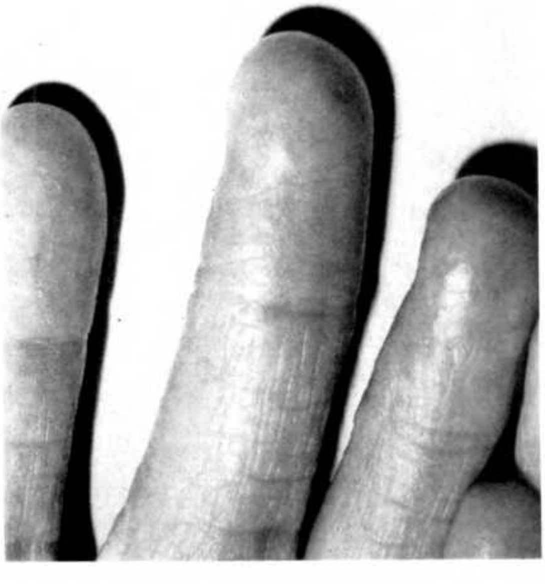

FIGURE 2. Smooth Fingertips.

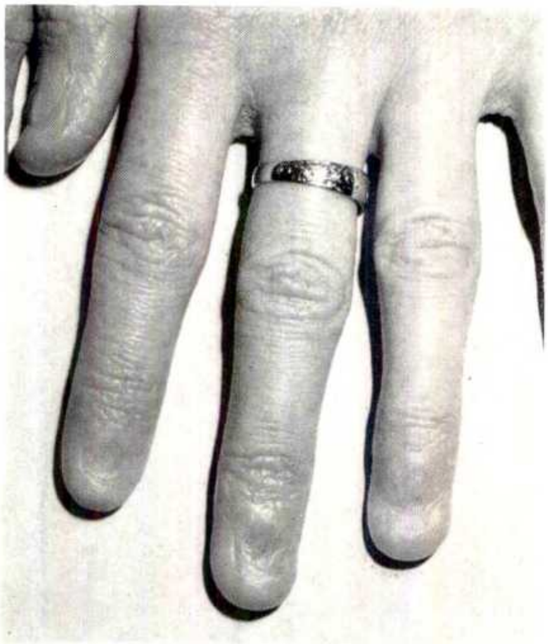

FIGURE 3. Anonychia.

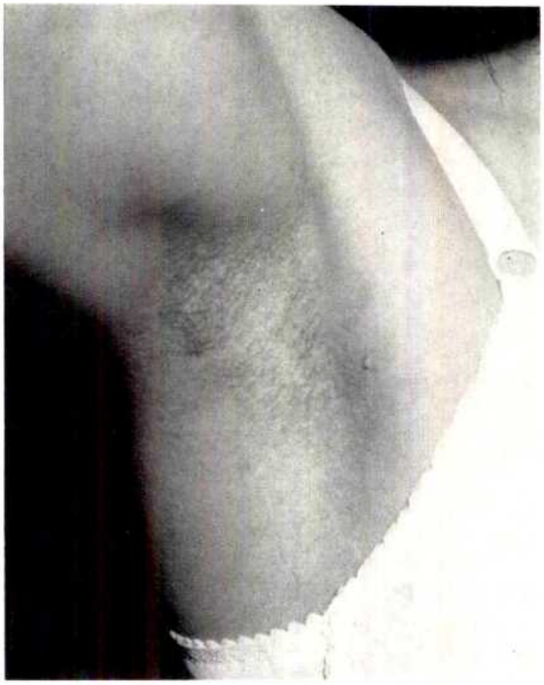

FIGURE 4. Hypo- and hyper-pigmentation of axilla.

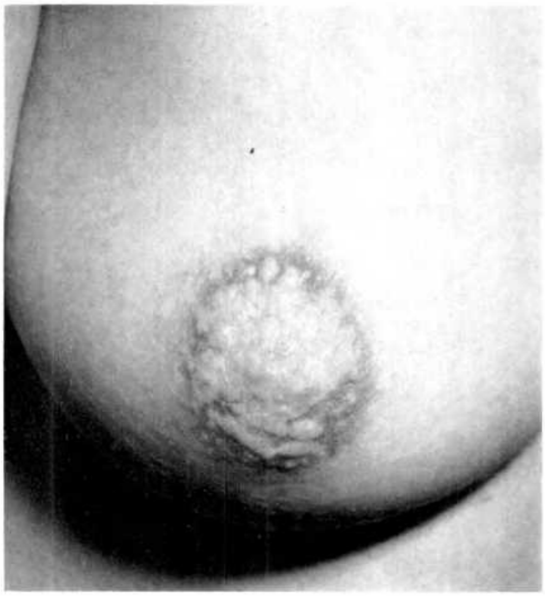

FIGURE 5. Breast showing lack of nipple pigmentation.

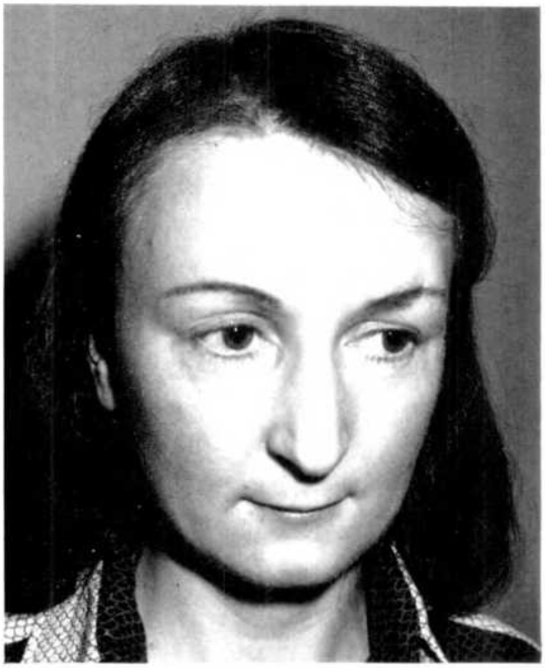

FIGURE 6. Facies.

was pigmentation at the outer edge of the breast areola (Fig. 5) but not of the nipple or areola itself. The above skin appearances had always been present according to the patient.

Scalp hair was coarse with some thinning in the frontal and vertex regions. The mouth, including the teeth, appeared normal. However, she had had numerous dental fillings and when a youngster, had been told that her teeth were unduly fragile. Her facies appeared normal apart from a distinctly hooked nose (Fig. 6).

Investigations including haemoglobin, blood film, total and differential white cell count, and X-rays of skull, chest and rib cage were normal. Palmar sweat testing with o-phthaldialdehyde (5% in xylene) was positive but the reaction was reduced.

The skin of her hands and feet has improved with 1% hydrocortisone cream and ointment and use of oily cream BP as a soap substitute.

FINDINGS IN OTHER AFFECTED RELATIVES

(II.5 aged 59 and III.4 aged 24 years)

II.2 was said to have been born without nails and to have shown a marked degree of scalp hair loss by the age of 21 years.

Facies

Like the propositus, both II.5 and III.4 showed a normal facies apart from a rather characteristically hooked nose. Unaffected individuals III.1 and III.2, who were not examined, were said to have a normal looking nose like their unaffected father who was examined.

Scalp hair

II.5 showed widespread thinning and coarse hair with less thinning at the scalp periphery. The thinning had been marked since the age of 39 years but she felt that recently there had been some regrowth.

III.4 had severe alopecia in a male-pattern distribution; thinning was first noticed at the age of 16 years. His hair was strikingly coarse. It was reasonably adequate at the sides of his scalp.

Teeth

II.5. Dental clearance was performed at the age of 18 years because of caries.

III.4 always had fragile teeth with caries a problem.

Sweating

II.5. She doubted whether she had ever sweated although she gave no history of any discomfort through inability to sweat. She showed moist axillae, and the o-phthaldialdehyde test over a hypothenar area was weakly positive.

III.4. He thought that he sweated only over the trunk but he showed moist axillae, fingertip sweating and his hypothenar o-phthaldialdehyde test gave a reduced positive reaction.

Palmar and sole skin

II.5. The skin over the palms and soles was dry with distortion of epidermal ridges. Although she suffered from cracked palms when younger this had improved with age.

III.4 had dry somewhat hyperkeratotic palms and soles with distortion of epidermal ridges but finger and toe ridge pattern formation was clearer than in II.5 and the propositus. Scattered telangiectases were visible over the lateral soles, and pits were present on the palms.

Pigmentation

II.5. Mottled hyperpigmentation was present in the axillae, antecubital fossae, neck and groins, and there were a few skin tags in the axillae and hypo-pigmentation of the nipples.

III.4 showed hypo- and hyper-pigmentation of his axillae, groins and perianal region with well-defined patches of hyperpigmentation over both tibial tubercles. There was slight hypopigmentation of his breasts with nipples not clearly defined from surrounding skin.

Nails

II.5. Fingernails were absent apart from rudimentary nails (which grew) on her thumbs and left little finger. All toe nails were present but rudimentary.

III.4. Fingernails were absent apart from a rudimentary right thumb nail. All toe nails were present but were rudimentary and only those of the right big toe and left 2nd and 3rd toes were said to grow.

DISCUSSION

It seems likely that this family has a hitherto undescribed skin condition affecting ectoderm, and inherited as an autosomal dominant trait. Consistent features of the condition include congenitally absent or rudimentary nails, macular hyper- and/or hypo-pigmentation particularly affecting groins, axillae and breasts but sometimes elsewhere, diminished ability to sweat, distortion of epidermal ridges over palms and soles, and coarse scalp hair which becomes markedly thinned in early adult life. Although some of the features may be present in other genodermatoses, particularly anhidrotic ectodermal dysplasia but also dyskeratosis congenita, the condition described is clearly a separate entity. The mode of inheritance, facial appearance, pigmentation, and anonychia distinguish the condition from anhidrotic ectodermal dysplasia, whilst dyskeratosis congenita, which almost always occurs in males, is associated with widespread pigmentary and atrophic skin changes and leukoplakia in the mouth is a feature.

It remains to be seen whether the close similarity of the manifestations found in affected individuals of both sexes of this one family will be a feature of other cases described.

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Comment

ENDONASAL LOCALIZATION OF BLOOD BORNE VIABLE AND NON VIABLE PARTICULATE MATTER

Most of the mutilating granulomatous diseases encountered by dermatologists tend to involve the nasal mucosa. Endonasal localization of lupus vulgaris occurs in the majority of cases (Forchammer, 1911; Degos, 1953; Horwitz, 1966). Lepromatous leprosy usually involves the nasal mucosa; thus Dharmendra & Sen (1948) in a study of 4072 cases of leprosy found that 92% of nasal scrapings from patients with lepromatous leprosy were positive for acid fast bacilli. Job, Karat & Karat (1966) and McDougall, *et al.* (1975) found heavy bacillary infiltration and phagocytosis by endothelial cells and perivascular macrophages of the anterior septum and inferior turbinates. Whatever the route of inoculation in the experimental mouse, the nasal mucosa eventually became heavily infected (Rees, McDougall & Weddell, 1974).

Of 1238 bedouin patients from the Syrian desert seen by Hudson (1958) with bejel, 695 had mutilating lesions of the centre of the face. Similar cases with eroded noses were recently reported from Kuwait (Kanan, Abbas & Girgis, 1971). In mucocutaneous leishmaniasis the nasal septum and nasopharynx are frequently attacked and other important diseases similarly affecting the nose are syphilis, yaws, rhinosporidiosis, paracoccidioidomycosis and rhinoscleroma (Kanan & Ryan, 1974). Paraense (1952) and Kanan (1975) reported endonasal involvement in guinea pigs inoculated with Leishmania enriettii. Cream (1974) has noted septum perforation in several of his series of patients with cryoglobulinaemia. Nasal septal perforation has also been a subject of correspondence as an early sign of lupus erythematosus (Simpson, 1974). Wegener's granulomatosis and lethal midline granuloma are the best recognized forms of mutilating vasculitis affecting the nose. The reason why the nose is selected in this way is the subject of some debate. It is clearly important to find out why organisms that are so often disseminated by the nose should tend to collect and even thrive there.

The nasal mucosa as a portal of entry for micro-organisms has been investigated by many workers. McMahon (1933) showed that colloidal carbon particles in suspension cannot penetrate the intact ciliated or squamous epithelium of the turbinates of the rabbit in spite of repeated frequent instillation over a period of 30 min to 6 h. But, Linton (1933) demonstrated that a sufficiently virulent strain of streptococcus haemolyticus is able to penetrate the normal mucosa without assistance in guinea-pigs and rabbits, providing the dosage is adequate. The work of Cannon & Walsh (1937), however, showed that virulent staphylococci, when instilled intranasally into normal rabbits and guinea-pigs, do not pass directly through the epithelium of the upper respiratory tract but are aspirated into the lungs and thence enter the blood stream. The endonasal route is not thought by most authors studying the diseases mentioned above to be the most likely portal of entry.

In lupus vulgaris, although Dowling & Wetherly-Mein (1954) believed that a considerable number of the lesions follow primary exogenous implantation of tubercle bacilli in the skin, Horwitz (1959, 1966) believed that it developed frequently as a result of haematogenous dissemination, a primary complex being present in another organ. Chest X-rays of 201 patients with lupus vulgaris as the only active tuberculous disease showed that sixty-six of them (33%) had pulmonary calcifications and another eleven patients showed fibrotic lesions of tuberculous origin.

In leprosy irrespective of the portal of entry it has been shown repeatedly that at some point in the disease acid fast bacillaemia has been a significant feature (Rivas, 1912; Crow, 1912; Honeij, 1915; Hollman, 1916; Rhodes-Jones, 1963). Recently Drutz, Chen & Lu Wen-Hsiang (1972) have convincingly demonstrated acid fast bacillaemia in twenty-five out of thirty-two patients with leprosy.

In six of these with untreated lepromatous leprosy acid fast bacilli were continuously present at a concentration of 10^5/ml of blood both extracellularly and in polymorphs, monocytes and large circulating macrophages. They thought that lepra bacilli seem to enter the blood stream through invasion of skin arterioles, capillaries and venules. Furthermore, Rees's work (1966) in the experimental mouse adds further support to a blood borne origin for endonasal lesions in lepromatous leprosy. Kanan & Ryan (1974a) described experiments which suggest that endonasal localization is non-specific and that it occurs predominantly through the blood stream as in cryoglobulinaemia.

Kanan (to be published) injected colloidal carbon intravenously into rats and observed that besides its well recognized clearance by professional phagocytes in the liver, spleen, bone marrow, adrenals and pituitary, the anterior septum and inferior and middle turbinates were other sites for its clearance into the tissues. In further experiments on other animals the same findings were recorded. Using radioactive colloidal gold, Kanan (in preparation) quantified the deposition of this material and found that the nasal mucosa in these sites was one of the significant catchment areas. Studies using intravascular inoculation of Leishmania enriettii confirmed that endonasal localization was not confined to inert particles (Kanan, in preparation). In their study of the angio-architecture in the nasal mucosa of the dog, cat, rabbit, rat, goat, sheep and pig, Dawes & Prichard (1953) emphasized the great number and large capacity of the venules as compared with the arterioles in the protruding naso- and maxilloturbinals and the anterior part of the septum. In a pilot study in the rat nose and other animal noses, Kanan has shown that the venular capillaries in the subepithelial and interglandular areas of the lamina propria are lined by an attenuated endothelium with frequent fenestrations. He also found the basement membrane nursing these capillaries and the venules to be irregular and often porous. However, similar findings have been described by Cauna & Hinderer (1969) in the human nose.

The biological behaviour of the nasal mucosa towards blood borne particles seems to be connected with this peculiar architecture of its microvessels, especially in the anterior part of the nose. In our opinion this has evolved under the mixed influence of two major factors, the first being a free physiological transport across the vessel walls bordering hyperactive secretory epithelium and the second being the constant bombardment by the cool and often polluted inspiratory air stream resulting in relative chronic stasis. Kanan & Ryan (1975) reported enhanced trapping of blood borne particles in the catchment area of the nasal mucosa after exposure of the experimental animal to a cold environment. On the other hand Kanan & Silver (1974) using special microprobes have obtained relatively low oxygen tensions in the subepithelial layer of the anterior nasal mucosa of the goat. They also recorded lower tissue temperatures in the same areas using surface thermisters. The previous findings suggest that the biology of stasis probably favours silting out of blood borne particles. Some granuloma producing micro-organisms may even thrive in this cool and relatively hypoxic site. Some workers have emphasized that lepra bacilli grow abundantly in tissues with temperatures cooler than normal body temperature (Brand, 1959; Binford, 1956; Shepard, 1965) but the modus operandi has still to be resolved. Samuel *et al.* (1973) showed that human macrophages in culture favour the multiplication of lepra bacilli in them equally well at 37°C as at 33°C, irrespective of their source. On the other hand Purtilo *et al.* (1974) have demonstrated inhibition of lymphocyte transformation on cooling *in vitro* and hence proposed that cell mediated immunity is probably depressed in the cooler areas of the body where these organisms thrive. It was also emphasized by several workers that tissue oxygen tension is a decisive factor in organ susceptibility to a particular micro-organism such as the tubercle bacillus (Novy & Soule, 1925; Lurie, 1963; Chandler *et al.*, 1965). They found that hyperbaric oxygen can inhibit the growth of M. tuberculosis both *in vitro* and in the experimental animal. Karnovsky (1962) emphasized that oxygen uptake was considerably higher in alveolar macrophages than in peritoneal macrophages in a resting state. Phagocytosis was inhibited in both cells when glycolytic inhibitors were added to the system but a state of anaerobiosis suppressed phagocytosis in the alveolar macrophages only. Russell & Roser (1966) suggested that macrophages can derive energy facultatively from oxidative or glycolytic pathways as circumstances demand. The stage of phagocytosis at which energy is consumed is probably actual ingestion. Rabinowitch (1967) showed that initial adhesion was not energy dependent. Uptake of large particles or groups of small particles into large vesicles was shown to depend on cellular metabolism (Casley-Smith, 1969). However, the clearance of a colloid from the circulation is dependent not only on uptake by macrophages but also on the rate of leakage between endothelial cells and out of the circulation (Carr, 1968; Kanan, 1975).

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INTRADERMAL TRIAMCINOLONE ACETONIDE TREATMENT OF PSORIATIC NAIL DYSTROPHY WITH PORT-O-JET

SIR, In my earlier study of 109 dystrophic psoriatic nails treated with intralesional triamcinolone acetonide (T.A.) by means of the Port-o-Jet gun a primary cure rate of 80% was noted after a single infiltration (Bleeker, 1974). The high frequency of recurrence led subsequently to the practice of infiltrating the matrix region three times at intervals of 2–3 weeks.

During the course of 1972 and 1973, 92 of 400 psoriasis patients were found to have nail dystrophies involving a total of 753 nails all of which were treated. Of these, 569 have been followed up with sufficient thoroughness to form the basis for the study described below. As in the earlier study, use was made of the Port-o-Jet gun for deep intradermal infiltration of the matrix region because of the relative freedom from pain offered by this method compared with needle infiltrations. Three areas were infiltrated around the matrix with a total of 0·2–0·3 ml of T.A. (with the smallest middle piece affixed) at intervals of 2–3 weeks. The curative effect was noted for each individual nail at follow-up 2–3 months after the last infiltration and again during the spring of 1974. Appreciable differences in the curative effect in individual nails of the same patient were noticed and therefore nail changes were graded as follows: nails with damage to matrix only (pitting, destruction, ridging, etc.); nails showing onycholysis only; nails with damage to the matrix and with onycholysis; and nails showing marked thickening of the nail plate. The effect of treatment was rated in terms of: cure; improvement; or in status quo. The period of observation ranged between 5 and 20 months.

Results. A primary cure or improvement was noted in 197 of 225 nails with damage to matrix only, but there were eighty-six recurrences. Eighty-one of 155 nails with onycholysis only were either cured or improved but the changes recurred in forty-nine. Of the 167 nails with both damage to the matrix and onycholysis the changes in the matrix healed or improved in 155 and the onycholysis in seventy-eight nails. Only five of the nineteen excessively thickened nails showed improvement, and there were three recurrences subsequently.

Conclusion. The Port-o-Jet apparatus has proved to be very well suited for infiltration of the periungual tissues with T.A. in the treatment of psoriatic nail dystrophy. After intradermal treatment with T.A. around the matrix a primary cure or improvement was noted in 73% of the nails of seventy-one patients with psoriatic nail dystrophy (569 nails) who had been observed from 5 to 20 months, but there was a recurrence rate of 46% after the period of observation. The nails affected by onycholysis showed a somewhat poorer cure rate and a higher recurrence rate than those with damage only to the matrix or damage to the the matrix combined with onycholysis.

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REFERENCE

BLEEKER, J.J. (1974) Intralesional triamcinolone acetonide using the Port-o-Jet and needle injections in localized dermatoses. *British Journal of Dermatology*, **91,** 97.

INFECTIONS IN DIABETES

SIR, I was interested in the review by Savin (1974) of infections in diabetes mellitus as I had carefully considered this problem when I wrote Recent Advances of Dermatology with Goldsmith in 1954. Like Savin, I found the evidence, which fortunately for me was much more limited in those days, conflicting and could not come to any firm conclusion.

My own experience with staphylococcal infections was that despite testing the urine in large numbers of patients with boils, one hardly ever found sugar; on the other hand, such a finding was not uncommon in those with carbuncles. Based on this and other evidence, I suggested tentatively that whilst boils were not more common in diabetics than in normal people, in the diabetics staphylococcal infections often took a more severe form and might develop into carbuncles. Of course it is possible that diminished sugar tolerance was more readily produced by the severe infection than by a simple boil but the glycosuria was usually so gross as to make this explanation unlikely.

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REFERENCE

GOLDSMITH, W.N. & HELLIER, F.F. (1954) *Recent Advances in Dermatology*, 2nd edn, p. 159. Churchill. London.

SAVIN, J.A. (1974) Bacterial infections in diabetes mellitus. *British Journal of Dermatology*, **91**, 481.

Book Reviews

Immunological Aspects of Skin Diseases. Ed. by L.FRY and P.P.SEAH (1974) Lancaster: Medical and Technical Publishing. Pp. 289. Price £7.50.

There is a great need for a book on the immunological aspects of skin diseases, and it is with regret that the reviewer cannot recommend this book. It is 'primarily aimed at dermatologists', but most clinicians would be confused by the amount of detail and failure to assess the relative importance of the various findings described in some chapters.

The chapters cover several aspects of skin immunology in which there have been recent advances, but they are uneven in their presentation and content, as if each was written for a different group of readers. In the chapter on pemphigus and pemphigoid by W.M.Sams, nine of the eighteen pages have a detailed description of the immunofluorescence technique, though the account of the diseases in the remaining half of the chapter concisely assesses the conflictory findings and their possible significance. In contrast, the chapter on dermatitis herpetiformis by L.Fry, and P.P.Seah, is a detailed and mainly chronological analysis of reports from eight and a half pages of references. The clinical aspects are discussed in relation to coeliac disease and immunological abnormalities, particularly those of IgA, and of anti-reticulin antibodies first described by the authors.

The work however contains one chapter that is excellent; a model review for informed clinicians and pathologists. Stephania Jablonska and T.P. Chorzelski present a logical, clearly written account of lupus erythematosus that is critical of the evidence yet clear in its conclusions and recommendations. Throughout the review of the data of thirty pages of references, the authors impose a personal 'touch' indicative of considerable practical experience. Another good chapter is that on malignant melanoma by R.C. Nairn, who achieves a good balance between clinical immunology and laboratory technology. The immunological findings of stimulated T lymphocytes inducing cytoclastic changes in tumour cells, and antibodies limiting metastases, are related to the clinical events and briefly to treatment.

The chapters on vasculitis and on eczema are, to put it mildly, disappointing. The account of the allergic changes in vasculitis is confusing. It is implied though not stated that the Shwartzman reaction is an allergic change (page 207); it is an error to state that the main function of complement is to make holes in bacteria (page 194), when most bacteria resist this action of complement. The reference to IgG_1 and IgG_2 (page 199) should have stated that Voisin's studies were made on guinea-pigs, because the subclass nomenclature of IgG applies also to other species. The description of two different cell types in cellular hypersensitivity being 'almost entirely if not exclusively B lymphocytes and/or their plasma cell progeny', and the monocyte (pages 208 and 209) is wrong. Surely the authors refer to T lymphocytes, especially as they continue to discuss macrophage inhibition factor. A reference to 'neutrophil mediated hypersensitivity' is also misleading. As a result of a hypersensitivity reaction, neutrophils mediate tissue changes; they do not mediate hypersensitivity.

The chapter on eczema, particularly atopic and allergic contact eczema, is poorly integrated. The accounts of the clinical classifications and features are clear and concise, but the immunological descriptions are involved and not always relevant. In a chapter on eczema, the relevance of a historical account of the finding of IgE, and a description of *in vitro* anaphylactic passive sensitization, is doubtful to support the evidence of increased amounts of IgE in the serum of persons with atopic dermatitis. Incidently human anaphylactic IgG is described as short latency IgG, when the original author expressly described it as short-term sensitizing antibody.

The editors failed to prepare from this collection of individual reviews, an integrated account of immunological aspects of skin diseases. There are three separate accounts of the immunofluorescence technique (pages 3–11, 164–169, 195–198), two accounts of the sequence of complement activation (pages 47 and 194) and two accounts of delayed hypersensitivity in contact dermatitis (pages 218 and 251 *et seq.*). Moreover there is too much jargon and error, a few examples are as follows: Neutrophils 'to be chemotactically mobilised' (page 48); 'heterogenicity' instead of heterogeneity (page 50); 'the action of dapsone. . . . is empirical' (page 55); and perhaps the most inexcusable is, 'unresponsiveness may be located in the afferent phase of the immunological response' (page 249).

The chapters on lupus erythematosus and malignant melanoma are good. There are better descriptions and reviews of the other diseases elsewhere.

W.E.PARISH

Insects and Other Arthropods of Medical Importance. KENNETH G.V.SMITH (Ed.) (1973) London, Trustees of the British Museum (Natural History) Pp. xiv+561, 12 plates+217 text figures. Price £15.00.

This book is intended to replace *A Handbook for the Identification of Insects of Medical Importance*, by John Smart, the first of the four editions of which was published in 1943. It was originally designed for medical officers working in the tropical war zones of the Old World. Despite its limited objectives, many dermatologists found it useful. The present work is international in scope and although it gives priority to problems of identification, it ranges widely over ecological and medical aspects of entomology and provides an immense amount of fascinating information of the greatest interest to the clinician eager to solve the mysteries of his patients' inexplicable 'prurigo'.

The sixteen authors, who include the editor, are almost all members of the staff of the department of entomology of the British Museum (Natural History) and they write with the authority of acknowledged specialists. Even with the help of the keys and the admirable line drawings, the identification of some species could be made only by those with training in entomology. Nevertheless the drawings, photographs and descriptions are so good that even the practising physician should be able to place a suspect in the right genus. The clear accounts of the life history of insects and other arthropods of medical importance are also practical and helpful, and the editor has not forgotten that species that do not transmit dangerous diseases may cause great discomfort. The biting midges have a short section to themselves and so do the almost equally tiresome ones which do not bite. The growing company of mites associated with skin lesions in man receive some attention in a chapter by J.G.Sheals on the Arachnida: dermatologists would certainly like to see still more about mites in the next edition, and few would share this author's apparent reluctance to incriminate Cheyletiella as a cause of troublesome skin lesions in man, as recently published reports and unpublished experience have shown it to be.

The bibliographies which end each section of the book deserve special praise for their international range and their general biological and medical interest.

This beautifully produced volume is modestly priced for its size and quality, and certainly deserves a place in the dermatologist's library.

ARTHUR ROOK

Gut-und Bösartige Erkrankgungen der Vulva. Klinik und Histologie. Band I. H.GRIMMER (1974) Berlin: Grosse Verlag. Pp. 124, illustrated. Price £10.25.

This is the first volume of Professor Grimmer's work on benign and malignant affections of the vulva. He deals with eczematous conditions, and some miscellaneous cases of vulvitis, in the first half of the book; the second half he devotes to kraurosis vulvae, which he appears to consider as synonymous with lichen sclerosus et atrophicus. Photographs, mainly clinical and almost all in colour, take up almost as much space as text. The text is confined to notes on the clinical and histological features, each section ending with a brief list of references (not free of misprints), the whole terminating in an index.

The author, a dermatologist, sees the book as being of assistance to gynaecologists and dermatologists working in the field of 'gynaecological dermatology' and regards it as mainly an atlas of visual information. Photographs of the vulval area, particularly in inflammatory conditions, are sometimes disappointing in teaching and diagnosis. The use of colour paradoxically may compound the shortcomings; certainly many of these pictures are unhelpful. The colour varies considerably, often even between pairs of photographs from the same patient to show lesions before and after treatment. Some photographs moreover are oddly selected and might be positively misleading; thus there are a few purporting to show lichen sclerosus on the cheek and chin. There is an apparently capricious use of colour rather than black and white pictures to illustrate histological features.

Vulvitis erosiva circinata and vulvitis plasmacellularis, neither accorded much attention in English texts, are considered at some length. Their aetiology remains obscure, the former perhaps related to bacterial infection, the latter perhaps a reaction to many factors. It would seem premature to dignify the acute ulcerative vulvitis reported by Boyce and Valtey in 1971, and that attributed to salivary contact by Davis in 1971, as definite entities. The author is particularly interested in the technique of intralesional injections of a corticosteroid for leukoplakia and some other conditions and describes it in detail.

For the rest this book with its mainly orthodox and straightforward views on dermatoses will have no special appeal for the English reader. For readers of German, however, and on its own ground it will no doubt prove useful.

C.M.RIDLEY

Year Book of Dermatology 1974. Editors F.D.MALKINSON and R.W.PEARSON (1974) Chicago: Year Book Medical Publishers. Distributed in the U.K. and Europe by Lloyd-Luke (Med. Books) Ltd. 49 Newman St., W.1. Pp. 446. Price £8.

The literature reviewed in this Year Book represents material published up to December 1973. The quality set in previous years of succinct abstracting and informed editorial comment is well maintained in this volume by the same editors. There is no introductory review article this year which is rather surprising in view of the high standard of such articles in recent editions. The careful balance between clinical and investigative material makes this edition, like its predecessors, essential reading for those wishing to keep up to date with dermatology literature in general.

RODNEY DAWBER

British Journal of Dermatology (1975) **92,** 483.

News and Notices

Dr Eugene M.Farber, Professor and Chairman, Department of Dermatology, Stanford University School of Medicine has been selected as the 1974 winner of the $1,000 Mr and Mrs J.N.Taub International Memorial Award for Psoriasis Research.

Dr Farber has authored, or co-authored, over 50 publications that document the breadth and depth of the studies performed by the Stanford group. In addition, Dr Farber has encouraged others to do research in psoriasis. He heads the National Program for Dermatology Task Force on Psoriasis and is editor of THE PSORIASIS BULLETIN.

Dr Farber was selected by a six man committee and the announcement was made by Dr John M.Knox during the Psoriasis Symposium of the Academy. The award was established in 1957 by Carol and Henry J.N.Taub in honour of their grandparents, Mr and Mrs J.N.Taub. Their establishment of this program carried with it the hope that the award would be an incentive to develop a cure to alleviate one of the problems of mankind.

Skin Pathology Group

There will be a meeting of the group on the afternoon and evening of Wednesday 2 July in Cambridge. Members of the group and others who have a particular interest in skin pathology are invited to attend. Further details may be obtained from the Secretary, Dr R. Marks, Department of Medicine, University Hospital of Wales, Cardiff CF4 4XW.

Course in Investigative Techniques in Dermatology

The Welsh National School of Medicine, Cardiff. 23–25 June inclusive 1975.
This course is designed for Registrars, Senior Registrars and non-medical graduates involved in dermatology research, who wish to gain insight into modern methods for the investigation of skin disease. Both theoretical and practical aspects will be covered. Speakers will include Professors Ebling, Greaves and Shuster; Drs Bleehan, Cronin, Cunliffe, Hunter, Mackie, Marks, Ramsay and Wells. Course fee £30. Further details from: Dr R. Marks, Department of Medicine, University Hospital of Wales, Cardiff CF4 4XW.

The Royal College of Pathologists

A Symposium on Immunological Aspects of Skin Diseases will be held at The Royal College of Pathologists, 2 Carlton House Terrace, London SW1Y. 5AF., on Wednesday 21 May 1975. The Speakers include Dr J. J. Cream, Professor M. W. Greaves, Dr A. D. B. Webster, Dr P. P. Seah, Dr E. J. Holborow, Dr A. Bryceson, Dr G. L. Asherson and Dr A. R. Hayward. Further details can be obtained from the Secretary of the Royal College of Pathologists at the above address.

Bulgarian Dermatological Association

The Second Congress of the Bulgarian Dermatological Association with international participation will take place in Sofia on 2–4 October 1975. The Main Themes are; (1) progress in the therapy of skin diseases, including climatotherapy, (2) the epidemiology and treatment of syphilis, (3) miscellaneous papers, (4) case presentations. Further information may be obtained from: Professor Dr P. Mikhailov, Institute of Dermatology and Venereology, Academy of Medicine, 1 Boul. G. Sofiiski, Sofia 31, Bulgaria.

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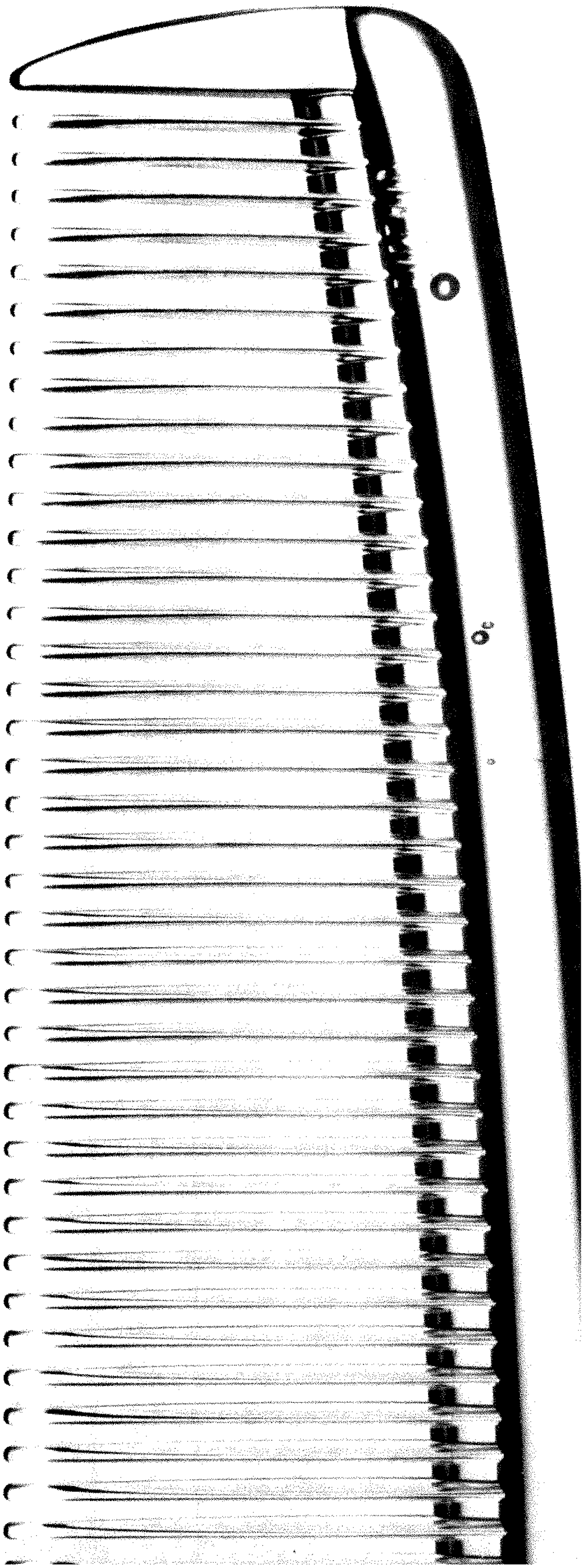

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

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kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
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microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations,* A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs.', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 92, Number 4, April 1975

CONTENTS

Clinical and Laboratory Investigations

PAGE

The Clinical Spectrum of Sweet's Syndrome (Acute Febrile Neutrophilic Dermatosis) — a Report of Eighteen Cases ... 363
D. A. GUNAWARDENA, K. A. GUNAWARDENA, R. M. R. S. RATNAYAKA AND N. S. VASANTHANATHAN

Cell Migration and DNA Synthesis in Organ Culture of Human Skin ... 375
P. M. GAYLARDE AND I. SARKANY

Prostaglandins and Cyclic AMP in Epidermis: Evidence for the independent action of Prostaglandins and Adrenaline on the Adenyl Cyclase System of Pig and Human Epidermis, Normal and Psoriatic ... 381
KENJI ADACHI, KUNIHIKO YOSHIKAWA, KENNETH M. HALPRIN AND VICTOR LEVINE

Smooth Muscle Antibodies and Alopecia Areata ... 389
ROBERT A. MAIN, ROSALENE B. ROBBIE, ELIZABETH S. GRAY, DAVID DONALD AND CHARLES H. W. HORNE

Serum Anti-tumour Antibodies and Auto-antibodies in Vitiligo ... 395
HAROLD WOOLFSON, OWEN A. FINN, RONA M. MACKIE, ALEX MCQUEEN AND RODERICK N. M. MACSWEEN

The Modifying Effect of Autologous Serum on Leukocyte Migration Inhibition by Liver Antigens in Systemic Lupus Erythematosus ... 401
P. HUGHES, SHIRLEY HOLT AND N. R. ROWELL

Emotions and Skin (II) — the Conditioning of Scratch Responses in Cases of Lichen Simplex ... 407
I. M. ROBERTSON, J. M. JORDAN AND F. A. WHITLOCK

The Areal Extent of Dermatophyte Infection ... 413
E. A. KNUDSEN

A Study of Chemical Light Screening Agents ... 417
T. M. MACLEOD AND W. FRAIN-BELL

The HL-A System and Psoriasis ... 427
J. C. WOODROW, V. K. DAVE, N. USHER AND JEANNETTE ANDERSON

Psoriasis in Children : A Short Review and a Survey of 245 Cases ... 437
ALLAN NYFORS AND KLAVS LEMHOLT

The Age-of-onset of Psoriasis and the Relationship to Parental Psoriasis ... 443
M. C. HOLGATE

Seborrhoeic Keratoses and Carcinoma of the Large Bowel: Three Cases Exhibiting the Sign of Leser-Trélat ... 449
K. LIDDELLL J. E. WHITE AND I. W. CALDWELL

M. Fortuitum amd *M. Chelonei* — Fast-growing Mycobacteria: A Review with a Case Report ... 453
JOANNA M. WARD

Case Reports

Wegener's Granulomatosis — Controlled by Azathioprine and Corticosteroids ... 461
J. M. WISHART

Anonychia with Bizarre Flexural Pigmentation — an Autosomal Dominant Dermatosis ... 469
JULIAN VERBOV

Comment

Endonasal Localization of Blood Borne Viable and Non Viable Particulate Matter ... 475
M. W. KANAN AND T. J. RYAN

Correspondence

Intradermal Triamcinolone Acetonide Treatment of Psoriatic Nail Dystrophy with Port-o-jet ... 479
J. J. BLEEKER

Infections in Diabetes ... 480
FRANK HELLIER

Book Reviews ... 480

News and Notices ... 483

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2. Archs. Derm. **86,** 608, 1962
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Nystavescent pessaries
a new answer to the growing problem
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Nystan Gel
applied to the surrounding skin
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nystatin 100,000 units

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PanOxyl acne gel

In this study *PanOxyl Acne Gel used alone gave outstanding results.

'93.3% patients improved and 91.1% liked the preparation'

In 78 patients already on systemic antibiotics, PanOxyl Acne Gel gave outstanding results.

'91% showed an overall improvement and 91% liked PanOxyl Acne Gel.'

'PanOxyl Acne Gel allows some patients to escape systemic antibiotics and their accompanying potential hazards . . . 35% of patients had their antibiotics stopped within 10 weeks of starting with PanOxyl Acne Gel. A further 37.2% had improved sufficiently to allow their antibiotic therapy to be reduced.'

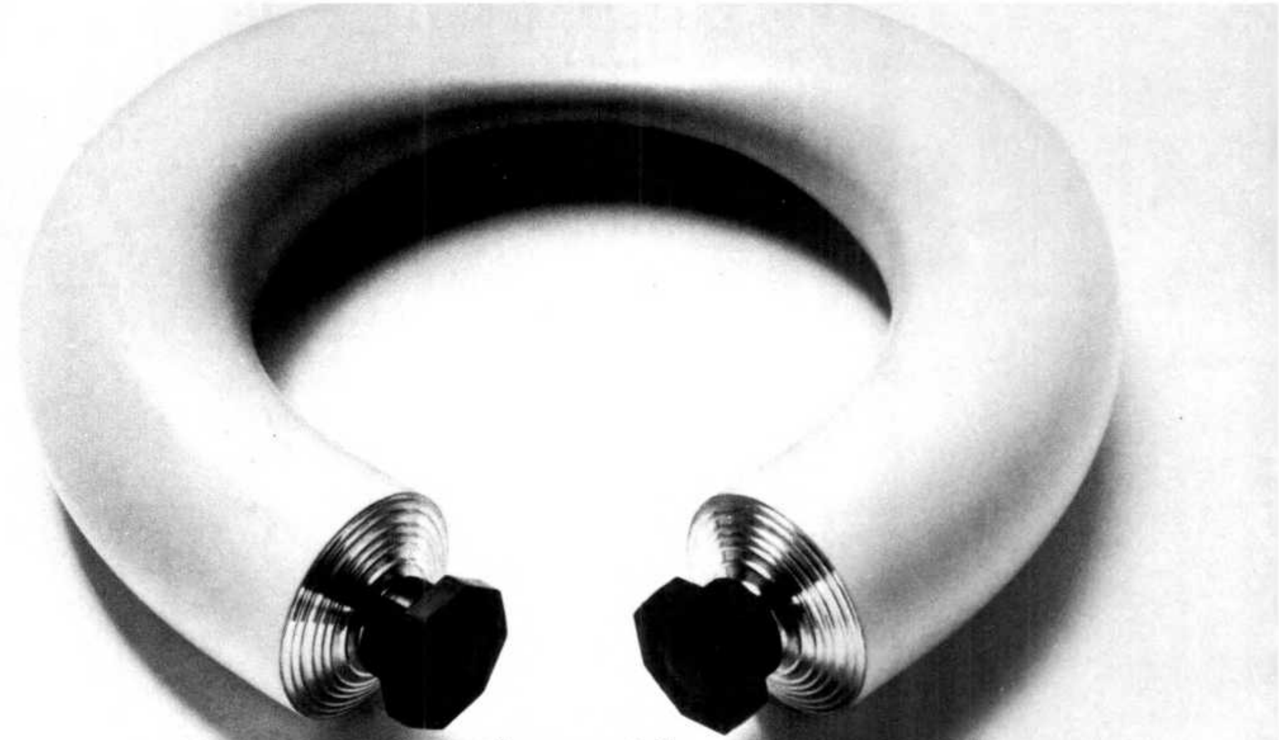
PanOxyl Acne Gel–first line treatment for acne.

PanOxyl Acne Gel – benzoyl peroxide 5% and 10% in gel base

Further information and patient instruction sheets on request

Stiefel Laboratories (UK) Limited
825 Yeovil Road Slough SL1 4JA

*Benzoyl peroxide gel in the treatment of Acne vulgaris
Brit. J. Clin. Prac. 1974, **28**, No 10, 379



Can one topical steroid combine both fluorinated effectiveness and hydrocortisone safety?

locoid

the non-fluorinated answer

Fluorinated steroids often have to be prescribed in order to achieve an effective control of a skin disorder. Unfortunately, in many circumstances, this may result in an unacceptable level of side effects such as skin atrophy, telangiectasia and striae. Locoid meets the need for effective treatment yet has been shown to be as safe as hydrocortisone.

As effective as the fluorinated steroids.

In an impressive series of double-blind trials[1234] Locoid proved to be as successful as Triamcinolone Acetonide, Betamethasone-17-valerate and Fluocinolone Acetonide in the treatment of both Eczema and Psoriasis.

As safe as hydrocortisone

Clinical experience confirms that Locoid, like hydrocortisone, is unlikely to cause side effects even when used on delicate skin areas such as the face.[5] Locoid does not contain fluorine and is metabolised into two naturally occurring substances, hydrocortisone and butyric acid.

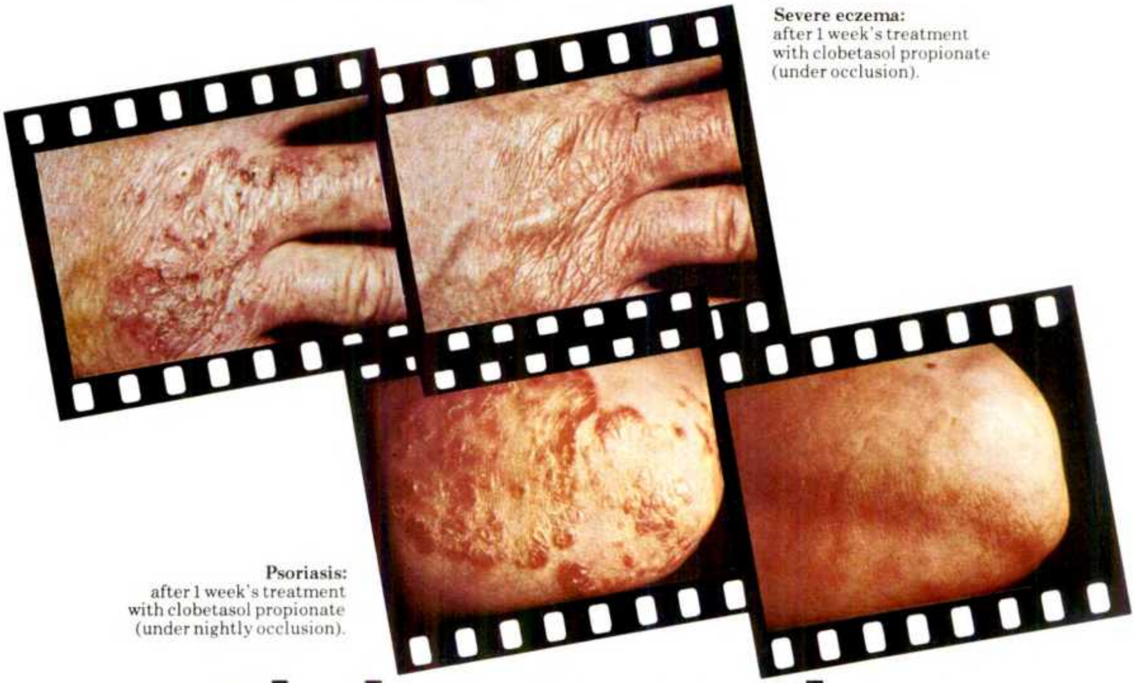
locoid

the logical first choice-
as effective as the fluorinated steroids
yet as safe as hydrocortisone.

Locoid is a trade mark for
hydrocortisone-17-butyrate

1 Alexander S. and Lyne C. Brit. J. Clin. Pract. (1973) 27177 2 Ashurst P. J. Brit. J. Clin. Pract. (1972) 23263 3 Polano M. K. et al Brit. J. Derm. (1970) 8393 4 Polano M. K. and Kanaar P. Brit. J. Derm. (1973) 8883 5 Sneddon I. Brit. J. Derm. (1973) 89505

Doctors have been known to stop prescribing Dermovate after just 7 days.



Severe eczema:
after 1 week's treatment
with clobetasol propionate
(under occlusion).

Psoriasis:
after 1 week's treatment
with clobetasol propionate
(under nightly occlusion).

It's clear to see why...

... Dermovate has proved itself so successful in the treatment of psoriasis – a searching test for any topical steroid.

It's not just in general practice that Dermovate (clobetasol propionate) is proving itself to be a top performer.

In clinical trials, involving double blind comparisons with other modern topical steroids, Dermovate showed itself to be significantly superior in the treatment of psoriasis.*

But not only in psoriasis has Dermovate proved superior.

In stubborn eczema and other recalcitrant dermatoses Dermovate clearly succeeds.

If you haven't prescribed Dermovate yet – do so today and see for yourself that Dermovate clearly succeeds in psoriasis and stubborn eczemas.

Dermovate

a top performer in psoriasis & stubborn eczemas

Glaxo

35mm photographs taken by a doctor in General Practice
*British Journal of Dermatology, 1974, **90**, 197
Dermovate is available as Cream and Ointment in 25 and 100 gram tubes

Literature on Dermovate (trade mark) is available from
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Snowfall

No fall

Calmurid Solution deals with scalp disorders – psoriasis, seborrhoehic eczema, seborrhoea, ichthyosis and atopic eczema. It keeps the scalp clean and free from crusts and scales by loosening and dispersing them. It also retains the natural moisture of the scalp. Note the nozzle on the bottle : it gets to the point – the scalp, not the hair. A sample awaits your request.
Side effects
possible transitory smarting
Composition
100ml solution contains
Carbamide (urea) B.P. 20% in a stabilising aqueous vehicle.

For scalp psoriasis,
seborrhoeic eczema,
seborrhoea

Calmurid Solution

Product Licence
No. 0009/0017

Write for full information to:

Calmurid is a registered trade mark.

Pharmacia (Great Britain) Ltd.
Paramount House,
75 Uxbridge Road,
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Telephone : 01-579 0102/7

Pharmacia

*Effectiveness in flexural dermatoses confirmed in trial at four major British Centres.
Water repellent
anti-candidal
anti-bacterial
anti-inflammatory
anti-pruritic

*** A trial conducted at four major British Centres.**

confirmed that successful treatment of skinfold dermatoses must overcome the adverse environment produced by local warmth and moisture, the risk of secondary infection and the increased sensitivity of flexural skin. Which is why the trial showed that "Topical corticosteroids alone are rarely curative in this type of eruption…"*

Timodine-the logical formulation for the treatment of ano-genital pruritus.

A water repellent to protect the skin against maceration and to allow the other active components to remain in direct contact with the affected area. Anti-candidal and anti-bacterial agents to combat secondary infection. A corticosteroid to relieve inflammation and pruritus.

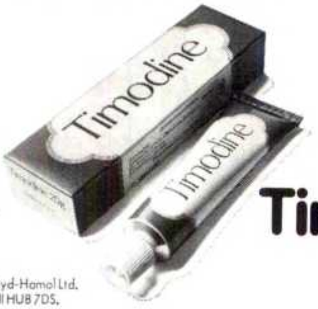
"This trial has shown that a cream containing a logical combination of active principles is highly effective in treating skinfold dermatoses."*

Timodine achieved marked success where other therapies had failed.

"Timodine produced an excellent or good result in 58 patients (79·4 per cent) with flexural dermatoses…Seventy-nine per cent of the 58 patients had previously failed to respond to a variety of other treatments."*

*Practitioner, (1974), **213,** 864.

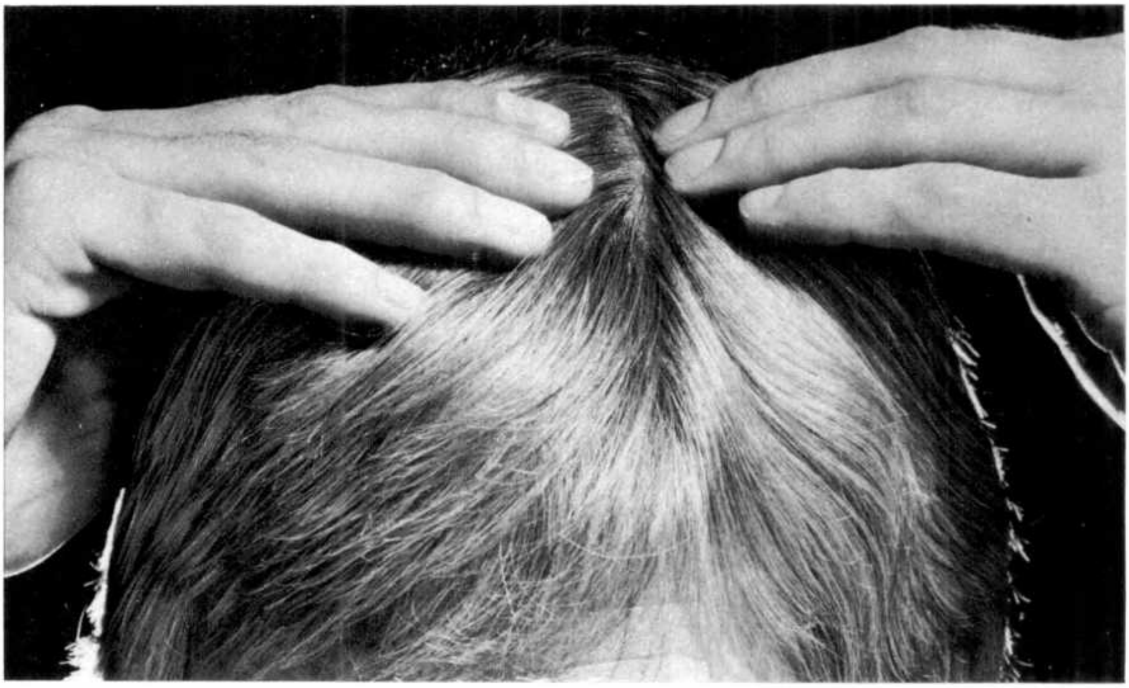
all the ingredients for the successful treatment of ano-genital pruritus.



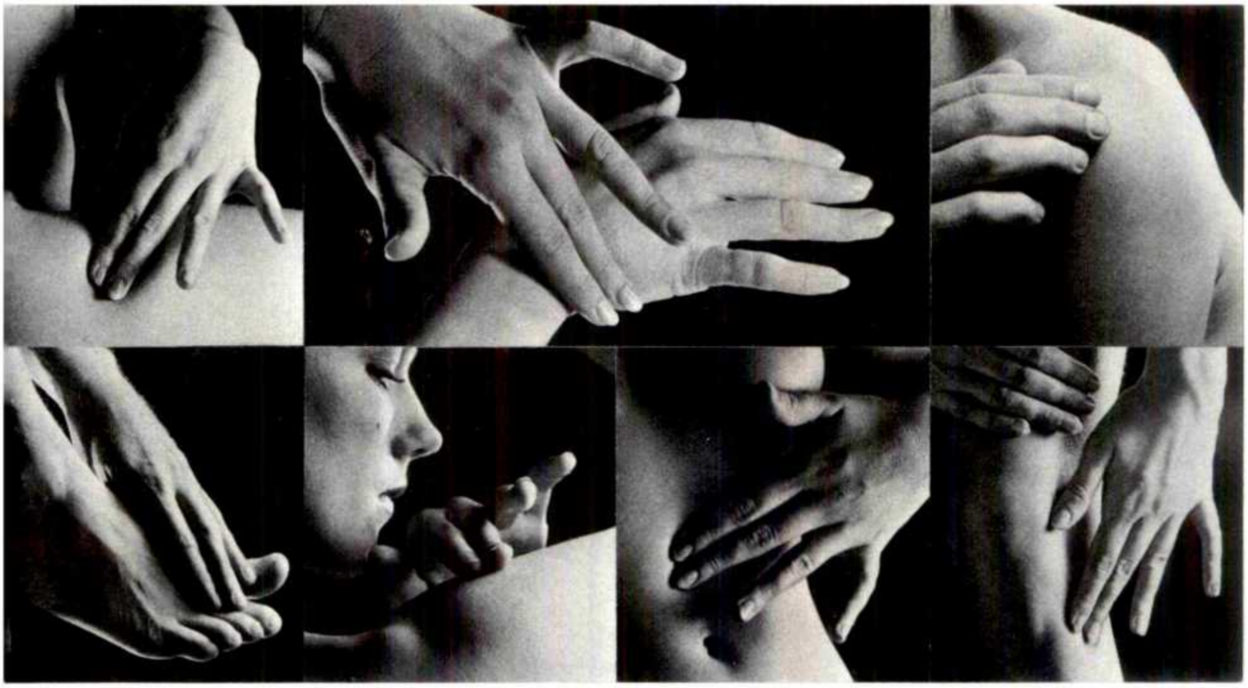
Timodine contains (w/w): Dimethicone 350, 10%; Nystatin, 100,000 i.u./g; Benzalkonium chloride soln. 0·2%; Hydrocortisone 0·5%. Timodine is a trade mark.
Basic N.H.S. cost 70p. Product Licence No. 1839/0001.

Timodine

Full prescribing information is available on request from Lloyd-Hamol Ltd.
A member of Reckitt & Colman Pharmaceutical Division, Hull HU8 7DS.



The proven success of Synalar Gel



is spreading.

'Synalar' Gel offers a preferable alternative to conventional creams and ointments in the treatment of inflammatory dermatoses on most body areas, whether they be hirsute or glabrous.

Economical in use. The clear, greaseless gel spreads easily to produce a thin, invisible film which creams and ointments cannot match and which patients find highly acceptable.

Successful treatment is less likely to be compromised by sensitisation or irritation. 'Synalar' Gel has been shown to be less irritant than many commonly used steroid creams and ointments[1] It is also free from sensitisers such as lanolin and parabens[2]

The superiority of 'Synalar' Gel over a variety of popular creams and ointments such as betamethasone 17-valerate has been shown by bioavailability studies[3,4] This is further confirmation of the outstanding clinical effectiveness of 'Synalar' Gel.

Synalar Gel *Available in tubes of 30g.*

'Synalar' (fluocinolone acetonide) is a trade mark.

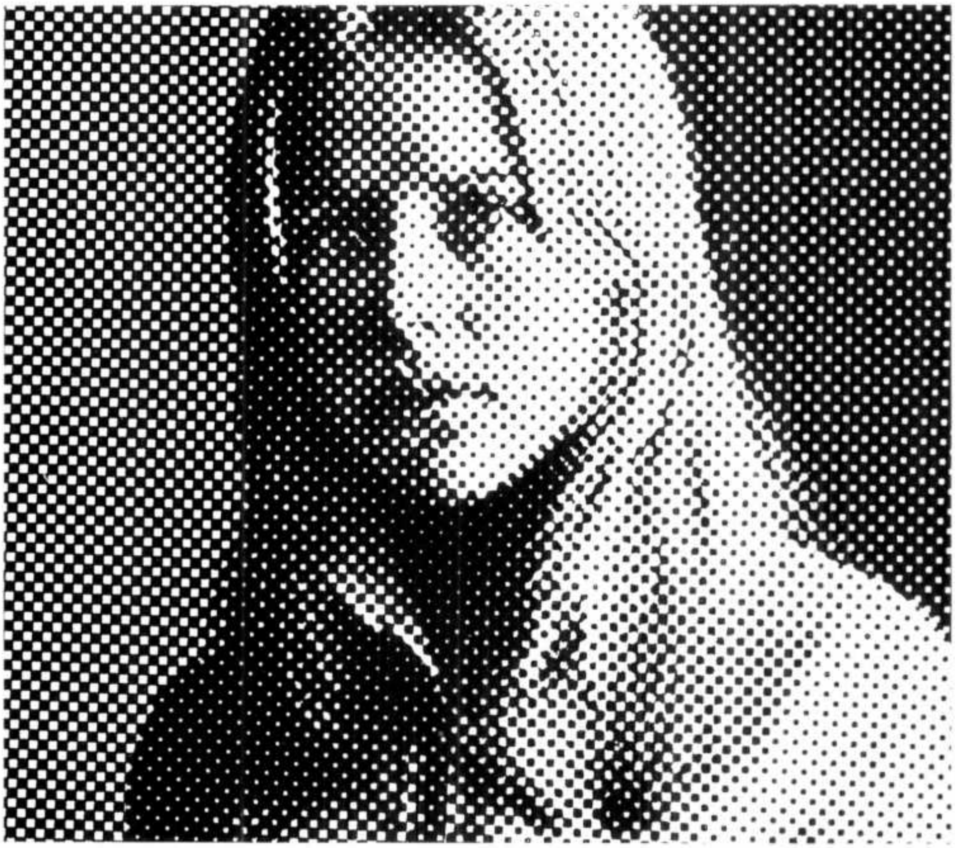
Nothing to see but results.

References:
1. Gaylarde & Sarkany. *Trans. St. Johns Hosp. Derm. Soc.* (1973), **59**, (2), 235–240.
2. Gaylarde & Sarkany. *Trans. St. Johns Hosp. Derm. Soc.* (1973), **59**, (2), 241–243.
3. Woodford & Barry. *Current Therp. Rsch.* 16, **4**, 338–345.
4. Woodford & Barry. *Brit. J. Derm.* (1974), **91**, 323–338.

Further information available on request.

ICI
Pharmaceuticals Division
Macclesfield
Cheshire SK10 4TF

THE QUEEN'S AWARD TO INDUSTRY
PH853



'Just a few spots' To the adolescent a desperate problem.

- "Ninety-one patients with acne completed a double-blind clinical trial comparing the effects of 'quinoderm', 'quinoderm hydrocortisone', and the astringent base used in the preparation of 'quinoderm' and 'quinoderm hydrocortisone'.

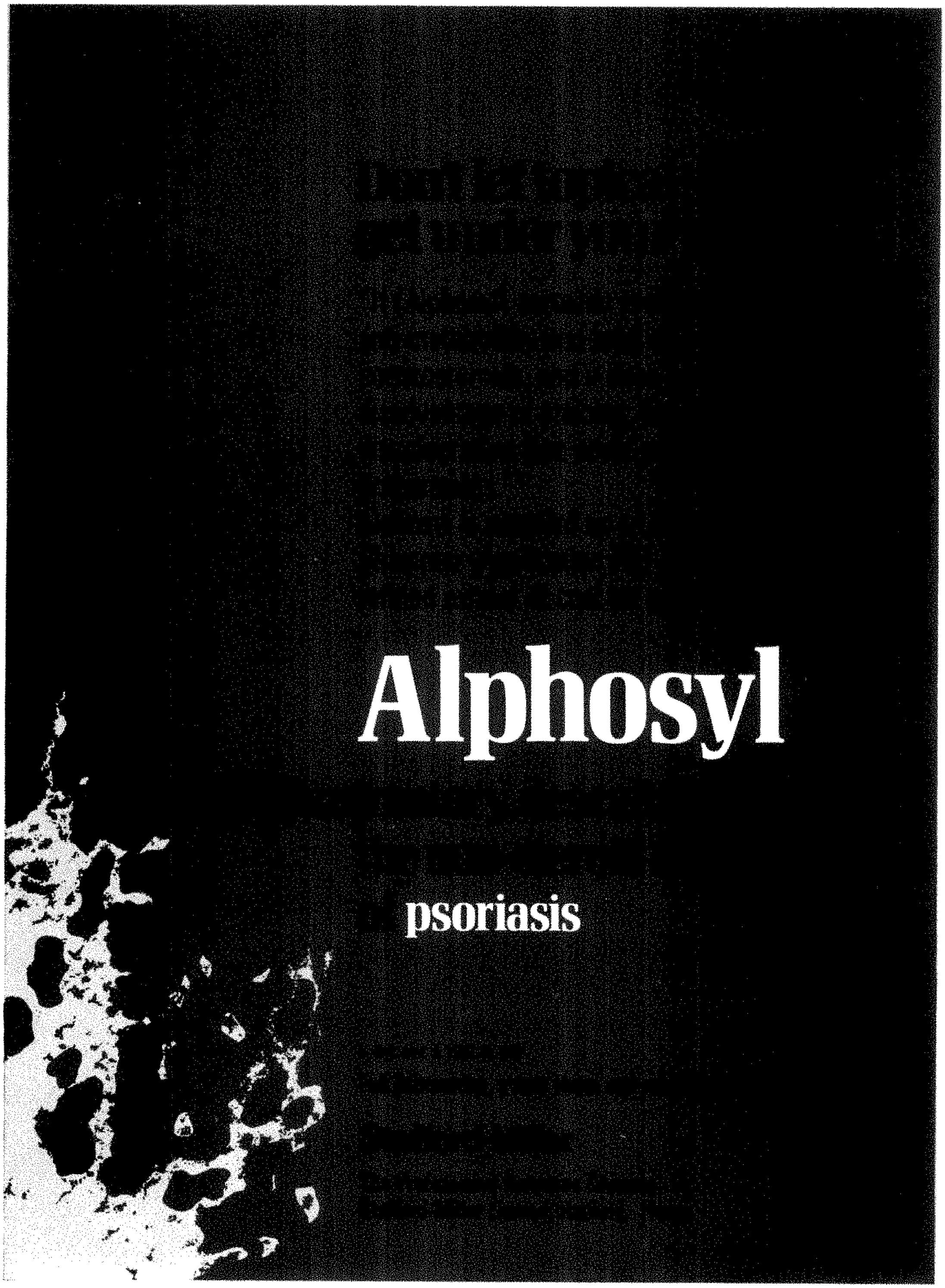
The response to 'quinoderm' and 'quinoderm hydrocortisone' creams was similar, with 80 per cent. improvement rate.

The response to the astringent base was 33 per cent."

When a patient like this asks for your help remember that clinical reports show that **'Acne Responds To Quinoderm'.** Available as Quinoderm with Hydrocortisone Cream 30G for initial treatment. Quinoderm Cream 25G for subsequent treatment.

- *'Practitioner' April 1972 pages 541-543*

For further information and literature
The Quinoderm Company, Oldham, OL8 4PB


Alphosyl
psoriasis

Some topical-steroid-responsive dermatoses are less responsive than others...

PROPADERM-FORTE CREAM

reduces the frequency of recourse to systemic steroids

When a patient with a steroid-responsive dermatosis is referred for therapeutic advice it can be expected that the condition has stubbornly refused to respond to at least one of the potent topical steroid preparations which are currently available.

These are the patients for whom Propaderm-Forte Cream offers a serious alternative to the initiation of systemic steroid therapy with its well-recognised disadvantages.

Propaderm-Forte Cream provides beclomethasone dipropionate BP 0.5%. In extensive clinical trials, employing the more usual concentration of 0.025%, it has proven to be as therapeutically effective as betamethasone 17-valerate[1] or fluocinolone acetonide[2]. Furthermore four clinical trials have confirmed that as a topical application it has no adrenal suppressive effect, as shown by normal plasma cortisol levels. In these trials[3,4,5,6] the steroid was applied for periods up to three weeks and to a total steroid dosage of 37.5mg.

Increased concentrations can, therefore, be applied in resistant cases with a high degree of confidence.

PROPADERM-FORTE CREAM

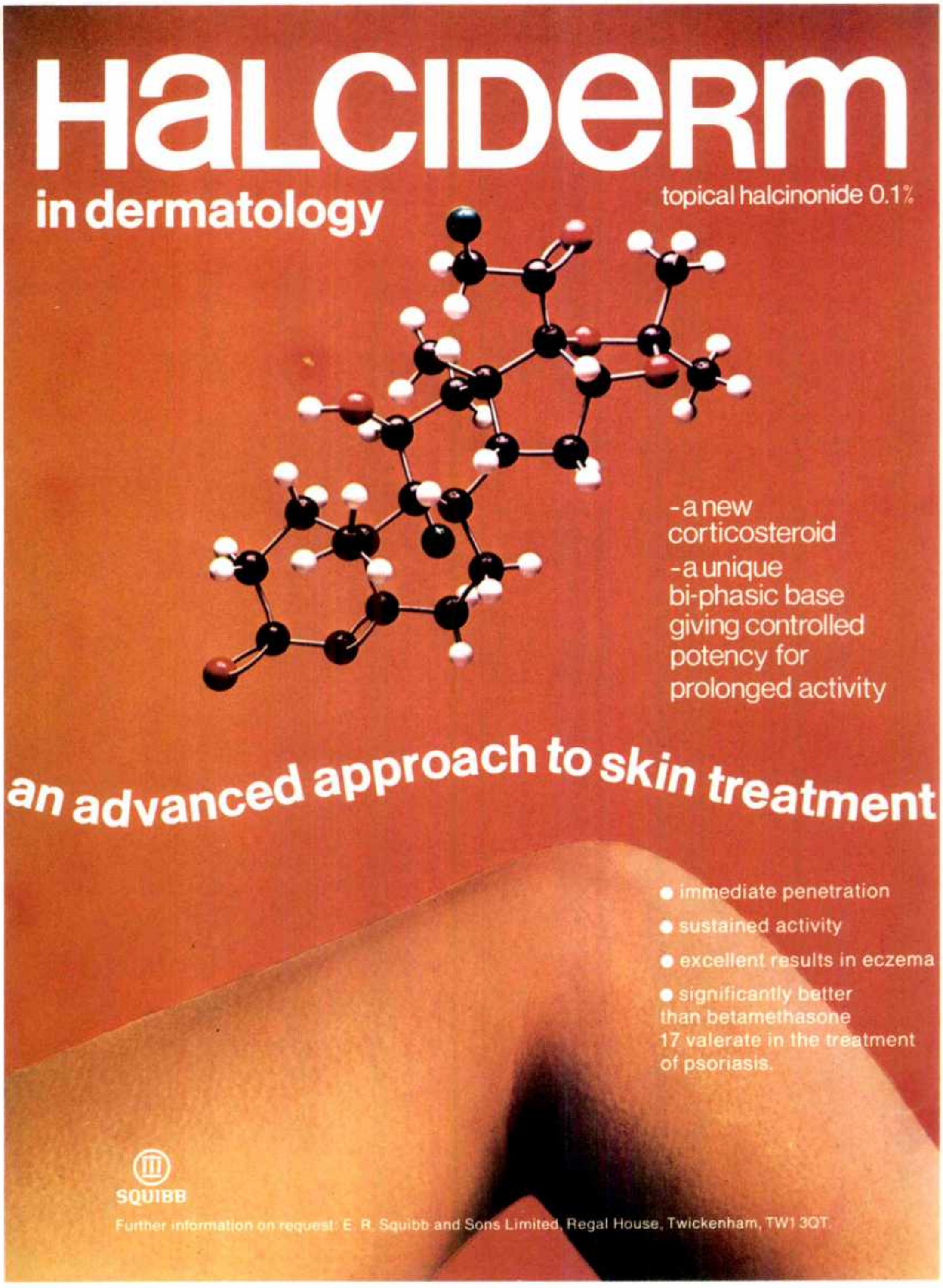
Effective topical steroid therapy in resistant dermatoses

References. 1. *Brit. J. Dermatol.*, 1968, **80**, 111; 2. *Brit. J. clin. Pract.*, 1970, **24**, 45; 3. *Int. J. clin. Pharmacol.*, 1973, **8**, 258; 4. *Brit. J. Dermatol.*, 1967, **79**, 487; 5. *Brit. J. Dermatol.*, 1968, **80**, 124; 6. *Clin. Trials J.*, 1973, **10**, 26.

Presentation. Propaderm-Forte Cream contains 0.5% beclomethasone dipropionate BP in a cream base. Available in tubes of 5g.

Beclomethasone dipropionate BP is also available in a concentration of 0.025% as: Propaderm Cream, Ointment and Lotion; Propaderm-A Ointment with aureomycin BP 3.0%; Propaderm-C Cream and Ointment with clioquinol BP 3.0%; Propaderm-N Cream and Ointment with neomycin sulphate BP 0.5%.

Full prescribing information is available on request. Propaderm is a trade mark of Allen & Hanburys Ltd.



HALCIDERM

in dermatology

topical halcinonide 0.1%

- a new corticosteroid
- a unique bi-phasic base giving controlled potency for prolonged activity

an advanced approach to skin treatment

- immediate penetration
- sustained activity
- excellent results in eczema
- significantly better than betamethasone 17 valerate in the treatment of psoriasis.

SQUIBB

Further information on request: E. R. Squibb and Sons Limited, Regal House, Twickenham, TW1 3QT.

"Pruritus ani and vulvae is a symptom that can be so annoying, embarrassing, and intractable that sufferers are willing to undertake any treatment to get relief."[1]

'Vallergan'

has so many benefits

antipruritic

'Vallergan' relieves the itching and so stops the scratching. 'Vallergan' reduces the need for any form of topical medication including corticosteroids. 'Vallergan' helps prevent exacerbation and secondary infection.

✻ analgesic

'Vallergan' relieves irritation and pain.

✻ antihistaminic

'Vallergan' is most effective in urticaria and other allergic skin disorders.

✻ sedative

'Vallergan' promotes sleep at night.

✻ well tolerated

'Vallergan' is well tolerated by infants, children and adults, including the elderly.

✻ presentations

You can prescribe 'Vallergan' either as tablets or as syrup, available in two strengths.

'Vallergan' for itching, scratching, and irritation in all conditions at all sites, at all ages, whatever the cause

Reference 1 Olansky, S.; in *Current Therapy*; edited by H. F. Conn, London: Saunders, 1969, p.646.

Full information is available on request
'Vallergan' is a trade mark of
May & Baker Ltd Dagenham Essex RM10 7XS
for its preparations of trimeprazine tartrate.

1974

M&B May&Baker

A member of the Rhône-Poulenc Group of Companies

New British discovery makes topical hydrocortisone as effective as its fluorinated derivatives

A unique drug-delivery system

British research has devised a new way of influencing the bioavailability of hydrocortisone. Alphaderm incorporates a unique drug-delivery system* enabling hydrocortisone to penetrate the epidermis efficiently. Optimum rehydration is achieved without recourse to occlusive dressings, and as the pH of the preparation approximates that of normal skin, stinging and smarting are avoided.

Alphaderm is as effective as the fluorinated steroids

A recent double-blind trial[1] has shown Alphaderm to be at least as effective as betamethasone 17-valerate in the treatment of atopic eczema.
Another recent controlled trial[2] has corroborated these results in other types of eczema. In addition, Alphaderm was shown to be significantly preferred by the patients.

Alphaderm – a more natural treatment of chronic skin conditions

Because hydrocortisone is a naturally occurring hormone, Alphaderm is less likely to cause the hazards that may accompany long-term therapy with fluorinated products.
Alphaderm contains naturally occurring body substances.
Alphaderm does *not* contain lanolin, parabens or other preservatives.

*Hypertonic solution of urea is adsorbed onto an inert powder matrix. This is incorporated into a water-retaining base to produce a non-greasy cream with unique hydrating and penetrating properties.

1. Almeyda, J. and Burt, B.W., *Brit. J. Derm.* (To be published) 2. Harries, E. H. L. and Jacoby, R. H. (To be published)

Full information available on request.

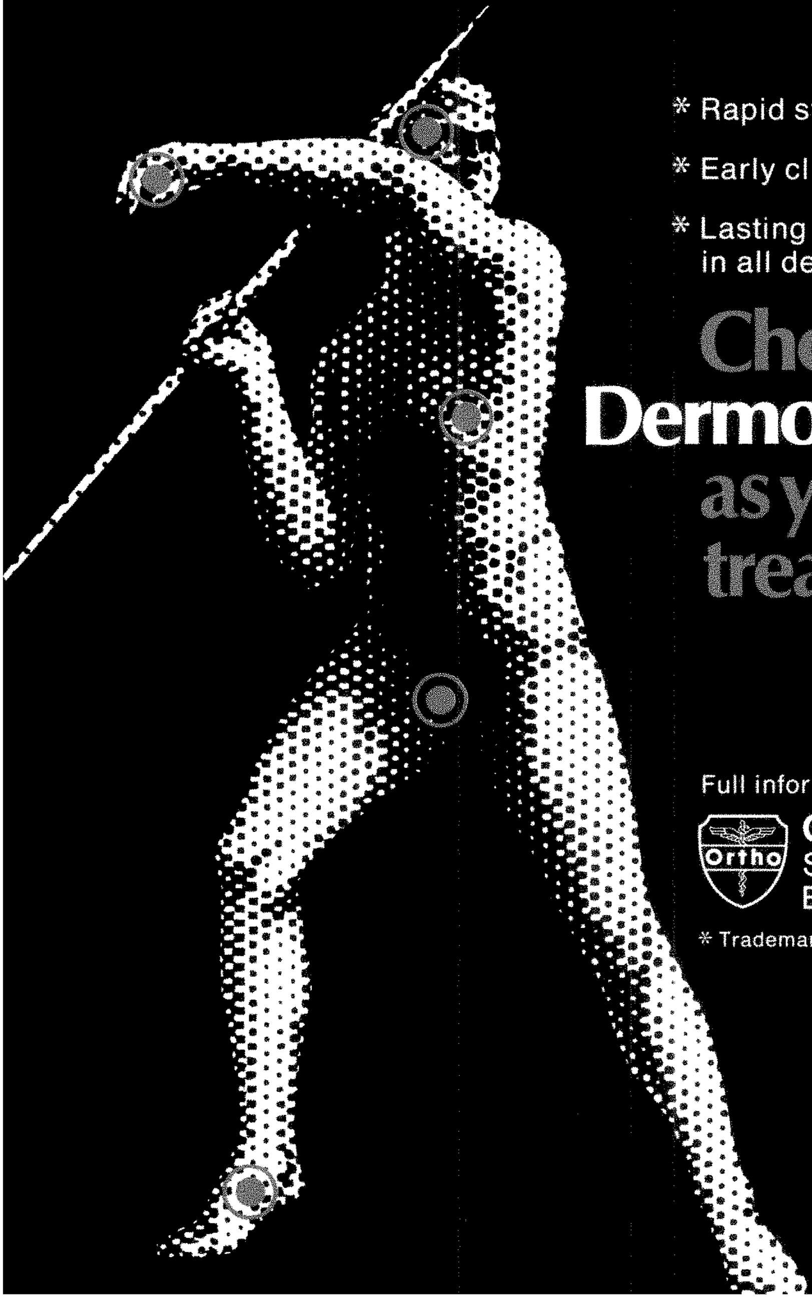
Eaton Laboratories, 125 High Holborn, London, WC1V 6QX

alphaderm

more natural and less hazardous in the treatment of non-infective dermatoses

Alphaderm contains 1% hydrocortisone in a special drug-delivery system containing urea: available in 30g tubes and 100g jars. 'Alphaderm' is a trade mark.

Athlete's foot – a recurring problem, responds rapidly to Dermonistat* Cream



- * Rapid symptomatic relief
- * Early clinical improvement
- * Lasting mycological remission in all dermal mycoses

Choose Dermonistat* Cream as your routine treatment

Full information on request:

Ortho Pharmaceutical Limite
Saunderton, High Wycombe
Buckinghamshire HP14 4HJ

* Trademark for miconazole nitrate 2%

Dermovate

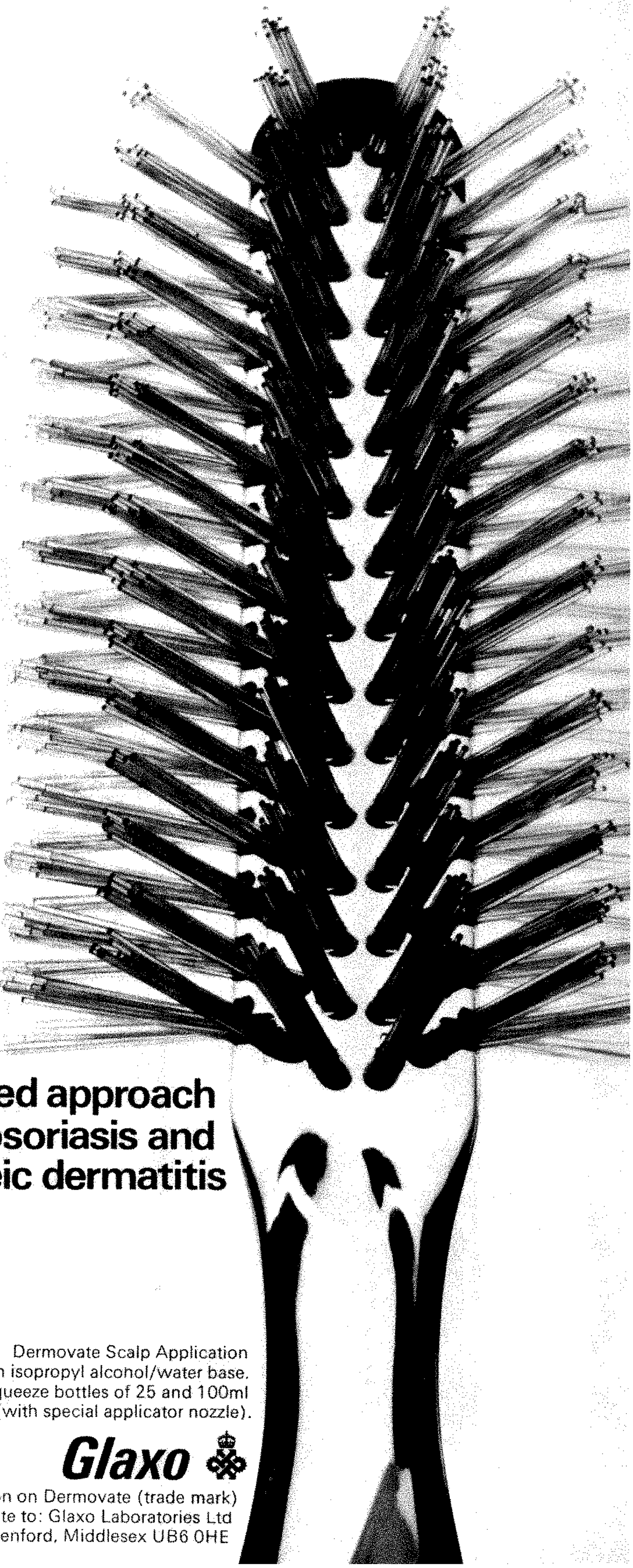
Scalp Application

Your patients will find there's nothing to show for it!

Treatment of scalp psoriasis and severe seborrhoeic dermatitis with Dermovate Scalp Application gives dramatic results.

No scaling, no itching and no mess! Dermovate Scalp Application is designed to deal with the difficult case. The long standing or recalcitrant condition. Dermovate Scalp Application contains clobetasol propionate which in clinical trials has shown itself effective in resolving these conditions, often succeeding where other therapies have failed, with improvement frequently being seen in just a matter of days.

Dermovate Scalp Application, in practical squeeze bottles with elongated nozzle, allows easy application direct to the scalp.

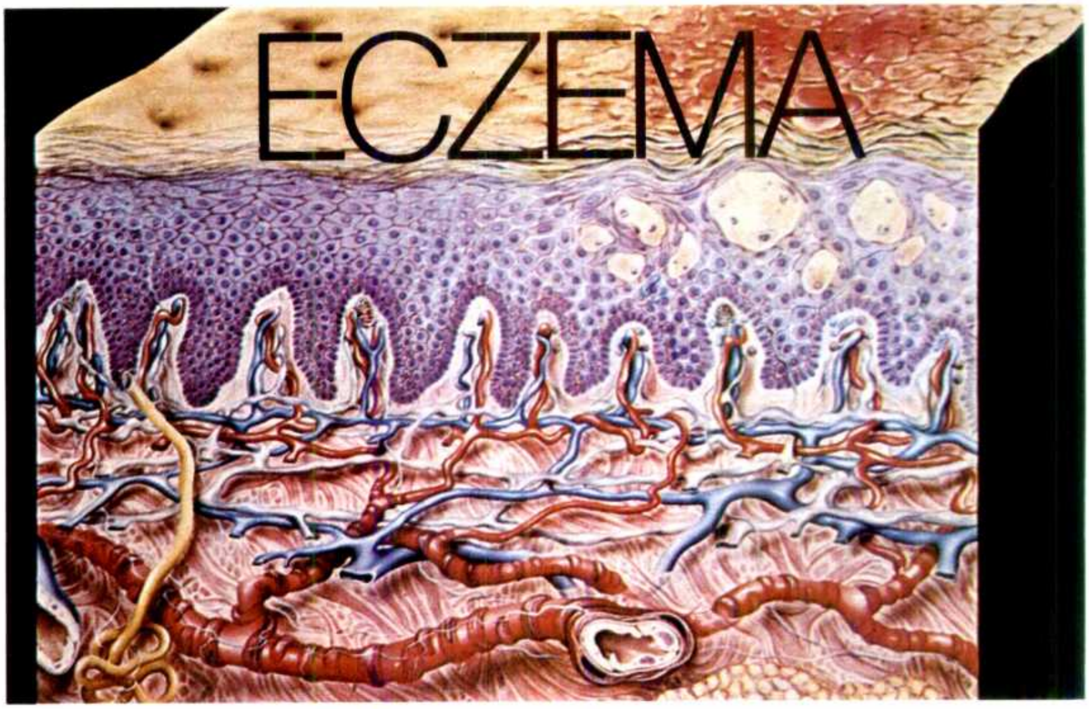


The clear headed approach to psoriasis and seborrhoeic dermatitis

Dermovate Scalp Application
—in an isopropyl alcohol/water base.
Available in plastic squeeze bottles of 25 and 100ml
(with special applicator nozzle).

Glaxo

For information on Dermovate (trade mark)
write to: Glaxo Laboratories Ltd
Greenford, Middlesex UB6 0HE



WHY RISK MAKING IT WORSE?

Many steroid vehicles include lanolin or parabens, despite recognition that both are potential sensitisers.
'Metosyn' contains neither and is free from the dangers of sensitisation associated with them.
'Metosyn' is fluocinonide formulated in a unique base; a combination offering significant advantages.

Once daily application - an economical régime which is as effective as betamethasone 17-valerate twice daily[2].

Proven to be more effective - against psoriasis, eczema and a wide range of both mild and severe inflammatory dermatoses.

One treatment for both wet and dry lesions

Preferred by a majority of patients[3]

Double-blind trials with over 1600 patients have confirmed that:

"... 'Metosyn' marks yet another advance in topical steroid therapy."[1]

Treat them to a clear skin with Metosyn–Once daily

'Metosyn' is a trade mark

Further information is available on request.

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References:
1) *British Journal of Dermatology*, (1972), **86,** 310.
2) *Wiener Medizinsche Wochenschrift*, (1973), **15,** 247-248.
3) *Current Therapeutic Research*, (1972), **14,** 259.

PH 851

British Journal of Dermatology (1975) **92**, 485.

Clinical and Laboratory Investigations

Albinism in Nigeria*

A CLINICAL AND SOCIAL STUDY

A.N.OKORO

Department of Dermatology, University of Nigeria Teaching Hospital, Enugu, Nigeria

Accepted for publication 13 September 1974

SUMMARY

A study of 1000 Nigerian albinos, all of Negro stock, showed various types of albinism with their different modes of transmission—oculocutaneous, ocular and cutaneous. The much higher incidence among the more settled communities in the south, compared with the more nomadic communities in the north, may be related to greater inbreeding tendencies in the south.

The sun and society are hostile to the albinos. Under the tropical sunshine, their melanin-deficient skin develops wrinkles, lentigines, actinic keratoses and epitheliomata from which they may die in early adult life or in middle age. Myopia and other ocular defects retard the progress of many albinos in school and they eventually drop out to seek disastrous menial outdoor occupations.

Registering albinos early in life, assuring their families that albino defects are confined to the skin and eyes, advising on protective clothing and sun-screening agents, correcting myopia, assisting with indoor occupations, and early treatment of actinic keratoses and skin cancer should help many albinos to attain social acceptance and a ripe old age.

Albinos are seen in every racial stock, but they stand out most strikingly among Negroes who are normally darkly pigmented. The existence of albinos in all the ethnic groups in Nigeria has long been recognized. Every major Nigerian language has a name for albinos: *Afin* (Yoruba), *Anyali* (Igbo), *Mbakara-Obot-Ikot* (Efik), *Eyaen* (Bini), *Ugobu* (Idoma), *Zebia* (Hausa). The Efik name is rather derogatory and means literally 'a white man from the bush'. Albinos are taunted from the cradle to the grave with such names as 'D.O.' (District Officer), a reference to the days when District Officers were Europeans.

References have been made to the incidence of albinism in Nigeria based mainly on casual observation (Barnicot, 1952; Clarke, 1958, 1959; Watkins, 1972) but no organized study to establish the real incidence had been undertaken. The present study was inspired by seeing epitheliomata destroying the faces, scalps, necks and shoulders of young albinos in their teens or twenties. Cancer of the skin in Negro albinos has been reported before (Clarke, 1959; Oettle, 1963; Marshall, 1964). The pitiful sight of young albinos driven by an uninformed society to menial outdoor work, under the pitiless tropical sun, was an associated reason for taking up this study.

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* Paper read at the meeting of the British Association of Dermatologists, London, July 1974.

MATERIALS AND METHODS

Starting from albino patients who came to the hospital with cancer of the skin, pre-cancerous keratoses, freckles, wrinkling of the skin or any other skin condition, attempts were made to trace all members of their families who were albinos.

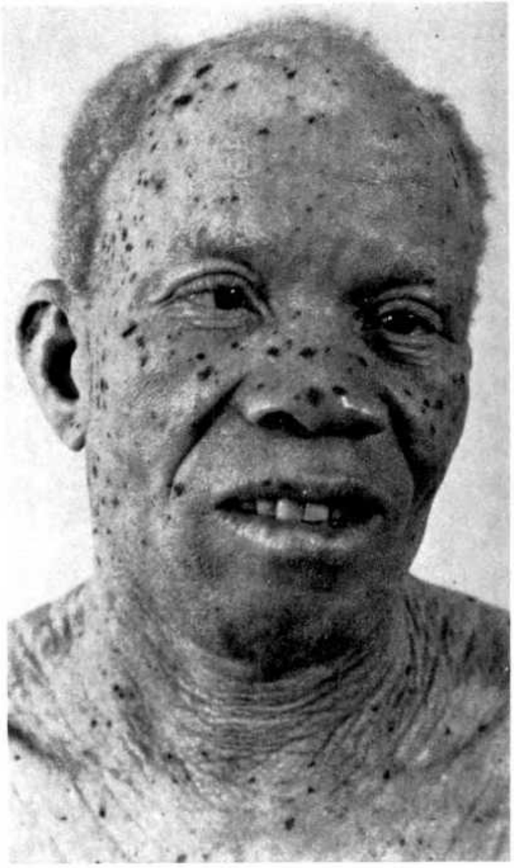

FIGURE 1. Thirty-year-old albino with ephelides and solar elastosis.

Then, through questionnaires and visits to various educational, health, and religious institutions, and to markets, more albinos were traced. The questionnaires explained briefly:

(1) The reason for the study (the delicacy of albino skin and its proneness to actinic damage).

(2) The greatest danger to albinos (skin cancer).

(3) The ultimate aims of the study (care and protection of albino skin, early detection, treatment, and prevention of actinic damage and its complications). The name for albinism was given in each of the major Nigerian languages for easier understanding of the questionnaires.

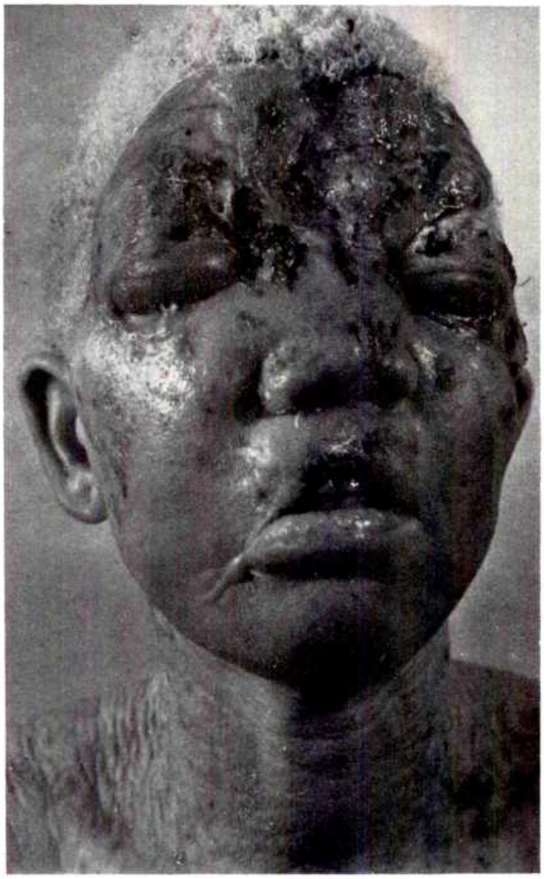

FIGURE 2. Twenty-eight-year-old female albino with squamous cell epithelioma.

A simple information slip on protective clothing (head-gear, clothes, and dark sun-shade glasses) was sent to each albino and, for those at school, an additional letter was sent to the school authorities recommending exemption of albinos from outdoor activities during the hottest and brightest hours of the day. For the albinos in public employment, their deployment indoors where possible was also recommended.

The first phase of the study covered the East Central State (where the author lives and works). The second phase covered all the twelve states in Nigeria. Albinos who came to our clinic with some dermatological problems or in response to our questionnaire were given a full medical examination, and were sent to the ophthalmologist for examination for ocular defects, and correction of amenable defects.

Histopathological examinations of albino skin and of the various lesions associated with actinic damage were carried out.

Factors in the physical and social environment which affect the survival of albinos, and therefore the incidence of albinism in the community, were recorded. Traditional beliefs about causation, and traditional attitudes to albinos were also investigated.

RESULTS

Nine hundred and eighty-nine albinos have been traced so far, 517 in East Central State and 472 in the eleven other states. Most of the albinos examined (95%) were of the oculocutaneous type characterized by absence or a markedly decreased amount of melanin in the skin, hair and eyes. Two per cent were cutaneous, with pale skin and hair but normally pigmented iris and retina. Three per cent were ocular, with pale iris and retina but normally pigmented skin.

Skin. At birth and in early childhood, albino skin has a delightful smooth texture but a sickly yellowish ivory colour. Its delicacy soon becomes obvious. The common bacterial infections of childhood (impetigo contagiosum, folliculitis, furunculosis and cellulitis) all look more severe than in normally pigmented children.

As the child grows and goes outdoors, the early effect of actinic irradiation (if it is not too intense) is a rather pleasant tanning of the exposed parts. But in time, the cumulative effect of years of exposure produces a motley of destructive skin lesions—sunburn, blisters, solar elastosis especially on the neck, arms and other exposed parts, ephelides (freckles), centrofacial lentiginosis, solar keratoses, chronic superficial ulcers and ultimately squamous cell epitheliomata and basal cell epitheliomata. The most exposed parts—forehead, ears, cheeks, nose, neck and shoulders usually suffer most. Sunburn with or without blistering, hypertrophic skin, ephelides and centrofacial lentiginosis are seen in all cases above 10 years of age. No albino over the age of 20 years was free from malignant or premalignant lesions. Keratoses and indolent superficial ulcers were present in 50%. Five of twenty albinos (3 males and 2 females) with squamous-cell epitheliomata treated (surgically) in the past 3 years, have died from extensive epitheliomata eroding the eyes, skull or large blood vessels.

Hair. The hair is a pale yellow colour in childhood and crinkly like normally pigmented hair. Later on, the hair may become deeper yellow, ginger or carrot-coloured. Dyeing it black produces a ridiculous and disappointing result because of the extreme contrast and the poor uptake of the black dye.

Eyes. The cardinal symptoms in oculocutaneous or ocular albinism are photophobia (all cases) and visual difficulties. Examination of the eyes revealed nystagmus in the great majority of cases, head nodding in very severe cases, and strabismus in a few cases.

The nystagmus and head nodding are due to lack of fixation rather than to neurological disorders. Absence or great paucity of pigment was seen in various parts of the eye—the iris, choroid and retina. The irides were pale or light-grey in childhood, becoming translucent or ash-grey in adult life. The fundi were pale or albinotic in all cases and the macula was improperly developed. Refraction tests revealed simple myopia in 90% of the cases and myopic astigmatism or hypermetropia in the rest.

The age distribution of the albinos showed a rapid drop in numbers after the third decade indicating the shortened life span of albinos. This is represented graphically (Fig. 3). Some of the older albinos may, however, have been missed in the questionnaire survey. Among 517 albinos traced in the East Central State, 89% are in the age range 0–30 years while 10% are in the age range 31–60 years. Among 472 albinos traced in the rest of the country, 92·9% are in the age range 0–30 years while 6·3% are in the age range 31–60 years. Among 1000 consecutive non-albino skin patients, 79·5% are in the age range 0–30 years while 20% are in the age range 31–60 years. A population distribution in a developing country (Lucas & Gilles, 1973) (Fig. 4) showed 78·3% of the population in the age range 0–30 years and 20·7% in the age range 31–60 years. This resembled closely the pattern in our non-albino patients.

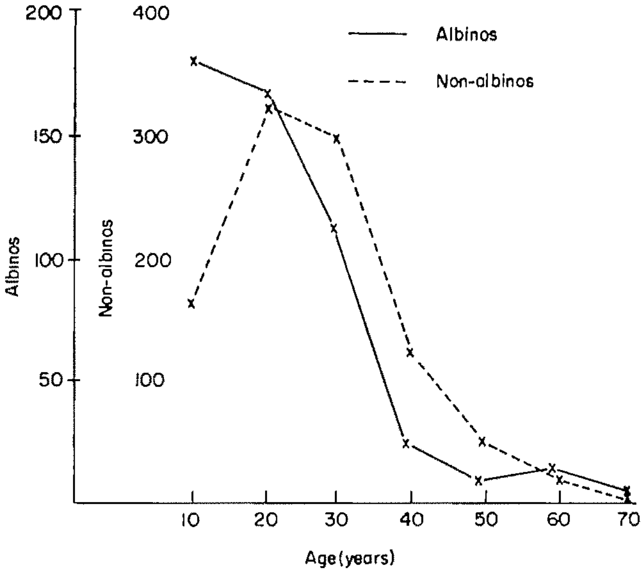

FIGURE 3. Age distribution of albino and non-albino cases.

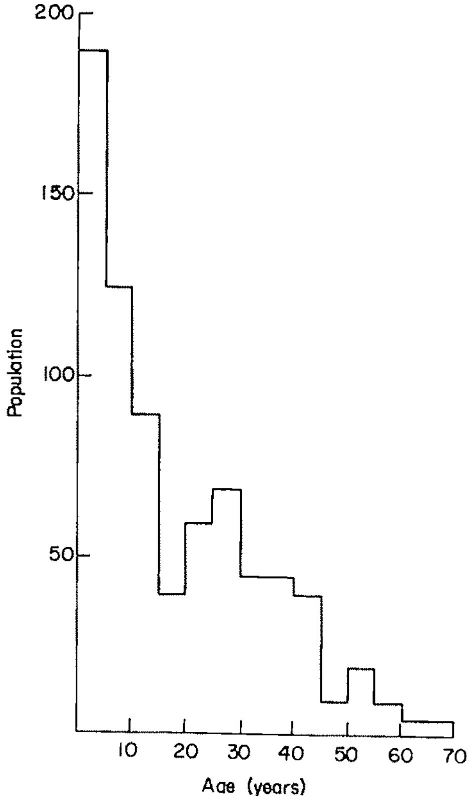

FIGURE 4. Population distribution in a developing country (Lucas & Gilles).

Sex distribution. Fifty-three per cent of the albinos were male and 47% female.

Albinos per family. In the East Central State, 63·3% of the affected families had one albino each, 26% had two albinos each, 7·7% had three albinos each, 2% had four albinos each, and 1% had five albinos each.

DISCUSSION

Nigeria is a vast country (area 357,000 sq. miles) inhabited by over 50 million people of almost exclusively Negro stock.

The map (Fig. 5) shows significant differences between the number of albinos in the six northern states where the population is more nomadic (72 albinos) and the six southern states where the population is more settled (917 albinos).

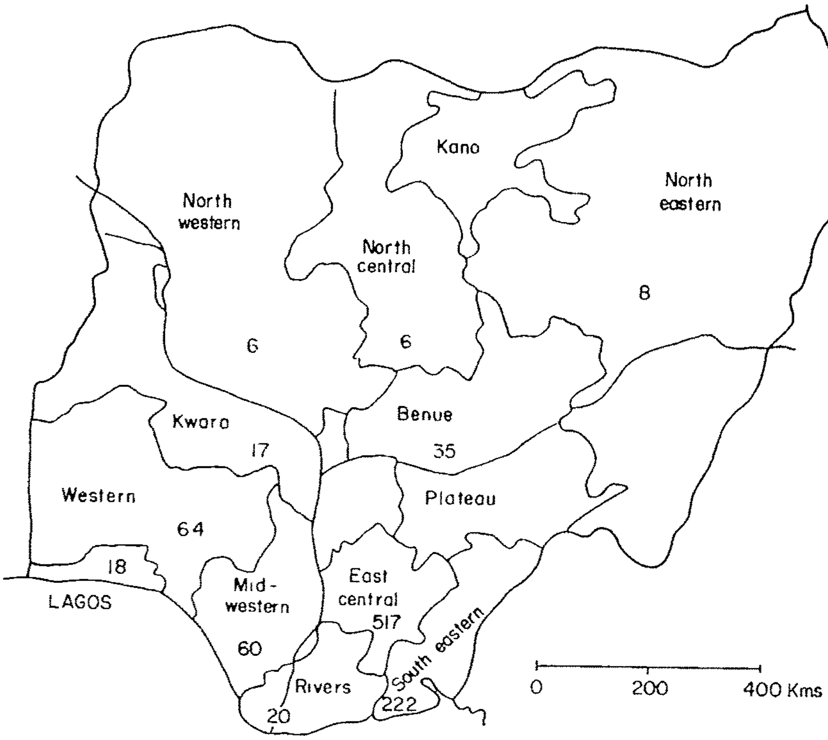

FIGURE 5. Nigeria: Distribution of albinos.

Inbreeding has been recognized as a major factor where there is a high incidence of albinism. The Cuna tribe of Indians on San Blas islands in Panama, inbreeding in isolation, have the highest incidence of albinism in the world—sixty-three per 10,000 (Keeler, 1970). The greater inbreeding tendency among the ethnic groups in Southern Nigeria may account for the higher incidence of albinism among them. Inbreeding, not in the sense of deliberate consanguineous marriages (though some do take place), but in the sense of marriage within the same town or clan, is very common in Southern Nigeria. This inbreeding has been encouraged in order to perpetuate some assumed superior qualities in the clan, not knowing that it also encouraged the perpetuation of albinism and probably of other recessive genes as well.

Nigeria, lying in the torrid belt from 4° to 14° above the equator, is sunny throughout the year. The role of solar radiation in the development of skin cancer has long been recognized (Unna, 1894; Findlay, 1928; Urbach, 1969). It is no surprise, therefore, that the majority of albinos, lacking the protection which melanin offers, develop skin cancer during their second, third or fourth decades, and succumb to it if no medical help is available.

Traditional attitudes

Nowhere in the traditional views on albinism is it believed to be associated with inbreeding. Rather, weird aetiological theories range from punishment from the gods, to conception during menstruation, or to seeing frightening sights during pregnancy.

Albino children are seldom as treasured as normal children because the inherent delicacy of albinos is well known.

Social life is very difficult for albinos. Neither their relations nor anyone else would agree that most albinos are normal in every respect except for the absence of pigment in their skin, hair and eyes. Albinos are taunted at home and in school. The less enlightened the society, the worse it makes life for the albinos. Many albinos drop out from school partly because of uncorrected refractory errors and partly because of discouragement. Reasonable employment is difficult for them to get, and marriage even more difficult, especially for the girls. The proportion of albino women who are married is very low.

Scope and prospects of the current study

This pilot study has spot-lighted only the tip of the iceberg. The problems of albinism and its study in such a vast and populous country are immense. They include, characteristically slow and incomplete questionnaire returns, ignorance, shyness, despondence, and reluctance to reveal the full extent of family involvement. A complete enumeration of all the albinos has not been achieved even in the East Central State which has been fairly extensively covered. It would therefore be unscientific to give the precise incidence of albinism at this stage. What has been recorded is the number of albinos traced so far. Only an exhaustive enumeration (to be carried out in future) can give the real incidence. As a pointer, among 20,000 skin patients seen in skin clinics over the past 6 years, 102 were albinos, giving an incidence of fifty-one per 10,000 in this group, albeit selected; but, from the figures available in this study, the incidence in the East Central State would be one per 15,000.

CONCLUSION

Albinos need protection against their major enemies—the neglect of an uninformed society, and the hostility of the tropical sun. Registering all albinos early in life, educating the society on the educability of most albinos with correctable ocular defects, and offering them encouragement and guidance through school and employment will help to restore them in society. The damaging effect of the sun can be minimized by protective clothing, sun-screening agents, and indoor occupations.

Regular examination of all albinos for early detection and treatment of the various premalignant and malignant lesions to which they are prone deserves to be included in the current anti-cancer campaign to which the medical world is committed. Finally, when further work has made the diagnosis of the heterozygous (carrier) state easy, then genetic counselling (avoidance of dysgenic marriages) should be advocated since, of the two cardinal variables in the albino-skin cancer association, namely inheritance and sunlight, the former is more readily amenable to control.

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Historical evidence for the genetic heterogeneity of pseudoxanthoma elasticum

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SUMMARY

Re-examination of published data from affected families with pseudoxanthoma elasticum (PXE) described in the literature between 1896 and 1968 supported the concept of clinical and genetic heterogeneity. There was clinical evidence for two autosomal dominant and one recessive type, although only a single patient with recessive type II was described. Examination of the individual pedigrees supported both autosomal dominant and recessive patterns of inheritance. The pooled genetic data were less helpful because they were so highly selected. (They had been reported in the literature.) Even so the dominant type II data fitted the hypothesis, whilst the type I and recessive groups were near the expected values.

The clinical properties of the groups corresponded closely to the recent British (Pope, 1973) survey. In general, the dominant type I group showed classical cutaneous changes, vascular complications were common and the chief ophthalmic complication was a severe choroidoretinitis. The dominant type II group, in contrast, had an atypical macular rash, or sometimes no rash at all, no vascular complications and very mild retinal changes. Choroidoretinitis was rare and peau d'orange patterns and prominent choroidal vessels common. The recessive group was of intermediate severity.

Four clinical types of pseudoxanthoma elasticum were clearly identified in a recent British survey (Pope, 1973). Autosomal dominant (Pope, 1974a) and autosomal recessive inheritance (Pope, 1974b) occurred and the methods of allocation to one or another group have been recorded. The various patterns can be identified despite some clinical overlap between the groups. The clinical features of 121 affected probands are summarized in Table 1a, grouped into the autosomal dominant and recessive types. These form the basis for the clinical criteria used to allocate newly detected patients to one or other of the groups. Similar criteria may also be used for retrospective allocation of patients previously described in the literature. Most previous authors have suggested autosomal recessive inheritance (Cockayne, 1933; McKusick, 1966) although undoubtedly autosomal dominant pedigrees had been described (Touraine, 1941; Cahill, 1957).

This paper re-examines and re-interprets other data in the light of the modern classification of the disease. The clinical criteria for allocation to one or another group are those summarized below and correspond to the clinical features set out in Table 1a. The results of re-allocation are provided for comparison in Table 1b.

CLINICAL CRITERIA

The families were allocated to one of four groups; autosomal dominant type I or II, and autosomal recessive type I or II according to the following criteria, which had previously been established for the British survey (Pope, 1973, 1974a, b).

TABLE 1a. Comparison of the clinical properties of probands from autosomal dominant and recessive groups examined in Britain (Pope, 1974b)

Characteristics	Dominant I	Dominant II	Recessive I	Recessive II
Number	12	52	54	3
Classical peau d'orange and flexural	12 (100%)	12 (24%)	40 (77%)	—
Macular	—	35 (70%)	7 (13·9%)	—
General increase of extensibility	1 (8%)	33 (66%)	2 (3·9%)	—
General cutaneous PXE	—	—	—	3 (100%)
Vascular disease				
Angina	7 (56%)	—	—	—
Claudication	7 (56%)	—	—	—
Hypertension	9 (75%)	4 (7·8%)	10 (19·7%)	—
Haematemeses	1 (8%)	2 (3·8%)	8 (15·8%)	—
Ophthalmic				
Severe choroiditis	9 (75%)	4 (7·8%)	18 (35%)	—
Angioid streaks	4 (34%)	24 (47%)	24 (47%)	—
Washed out pattern		6 (12%)	1 (1·9%)	—
Peau d'orange		4 (8%)	—	—
Prominent choroidal vessels		9 (18%)	—	—
Myopia	3 (24%)	24 (48%)	3 (5·8%)	—
High arched palate	—	27 (54%)	3 (11·9%)	—
Blue sclerae	1 (8%)	21 (41%)	5 (9·8%)	—
Loose jointedness	—	18 (35%)	3 (5·8%)	—

(*a*) *Dominant type I*

This type shows a flexurally distributed peau d'orange pseudoxanthomatous rash, severe vascular complications, such as angina, coronary artery disease and claudication, and a severe degenerative retinopathy, with early blindness.

(*b*) *Dominant type II*

This type, which is much less severe, typically has a canary-yellow macular rash, minimal vascular symptoms and mild retinal changes with prominent choroidal vessels. Increased cutaneous extensibility, blue sclerae, a high arched palate and myopia are also signs.

(*c*) *Recessive type I*

This resembles the dominant type I group although vascular and retinal degeneration are much milder. Haematemeses are common, especially amongst affected females.

TABLE 1b. Clinical characteristics of individuals described in the literature

Characteristics	Dominant type I	Dominant type II	Recessive
Number	41	32	56
Classical cutaneous	40 (99%)	11 (33%)	42 (75%)
Solar elastosis	4 (10%)	—	—
Macular or slight change	2 (5%)	20 (60%)	1 (1·8%)
Hyperextensible skin	—	5 (15%)	—
Very extensive change	—	—	1 (1·8%)
Vascular disease	12 (30%)	—	2 (3·5%)
Intermittent claudication	5 (12%)	—	3 (5·2%)
Cerebrovascular disease	2 (5%)	—	1 (1·8%)
Hypertension	5 (12%)	—	3 (5·2%)
Haematemeses	3 (7·5%)	2 (6%)	3 (5·2%)
Ophthalmic complications			
Choroidoretinitis	23 (62·5%)	1 (3%)	23 (41·7%)
Angioid streaks	4 (10%)	5 (15%)	16 (28·6%)
Peau d'orange	—	10 (30%)	—
Salmon spots	—	1 (3%)	—
Prominent choroidal vessels	—	4 (12%)	—
Myopia	—	7 (21%)	1 (1·8%)
Miscellaneous			
Hypercholesterolaemia	2 (5%)	—	2 (3·5%)
Miescher's elastoma	1 (2·5%)	—	—
High arched palate	—	2 (6%)	—
Blue sclerae	—	1 (3%)	—
Marfanoid habitus	—	2 (6%)	—
Consanguinity	—	—	4 (6·9%)

(*d*) *Recessive type II*

This unique and rare variant shows generalized cutaneous laxity and infiltration without systemic complications. Only three families were detected out of some 140 examined in Britain (Pope, 1973, 1974b) and none could be found in the previous literature which this paper describes.

Genetic pattern

There was general correlation of clinical pattern with clinical type. Most, but not all, patients with the clinical signs characteristic of dominant types came from families with affected members in at least two generations, with all combinations of parent to child transmission. Patients with signs characteristic of recessive types all appeared in one sibship, except in the family of Loria *et al.* (1957) (Fig. 6), where an affected individual who married a relative had an affected offspring. Sporadic cases were allocated to a genetic category on clinical criteria.

CLINICAL DESCRIPTIONS

(*a*) *Autosomal dominant type I* (Figs 1 and 2)

Jones, Aldren & Bishop (1933) reported a man aged 44 years, who presented with a sudden blurring of vision. He had had cutaneous PXE since childhood and had an affected sister. Although his mother was reported as normal, she had not had a biopsy and her sister had classical cutaneous and ophthalmic

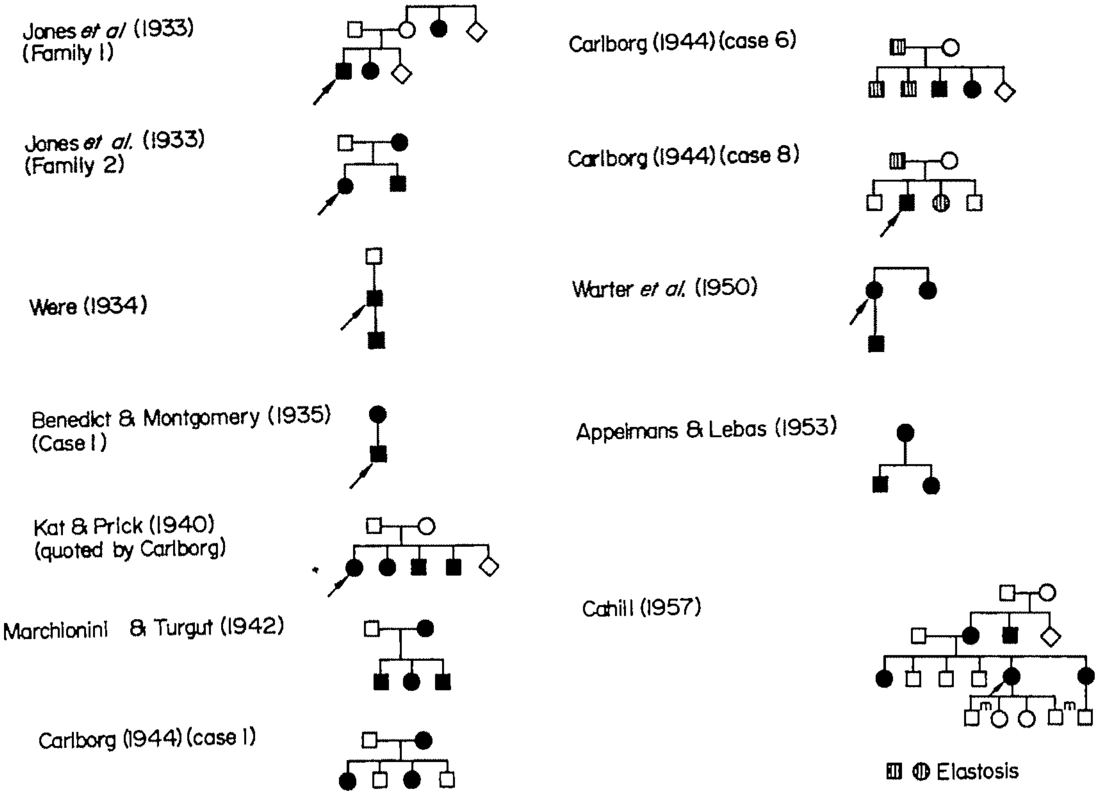
Jones *et al* (1933) (Family 1)
Jones *et al.* (1933) (Family 2)
Were (1934)
Benedict & Montgomery (1935) (Case 1)
Kat & Prick (1940) (quoted by Carlborg)
Marchionini & Turgut (1942)
Carlborg (1944) (case 1)
Carlborg (1944) (case 6)
Carlborg (1944) (case 8)
Warter *et al.* (1950)
Appelmans & Lebas (1953)
Cahill (1957)
m
m
Elastosis

FIGURE 1. Pedigrees of possible autosomal dominant type I families reported.

disease. This observation strongly implied that the mother was heterozygous. In the same paper, a woman aged 46 years was described who had classical cutaneous and ophthalmic PXE; her mother and brother both had a degenerative choroiditis.

Were (1934) described an affected father and son. No other family details were available.

Benedict & Montgomery (1935) described several affected patients. Case 1 was seen with a 40-year history of axillary cutaneous PXE. He then developed visual impairment and a degenerative choroiditis. His mother was found to have a similar choroiditis.

Hagedoorn (1939) described a 48-year-old woman with classical cutaneous disease, who, 2 years earlier, developed a right hemiplegia. Her father had died aged 63 years with gastrointestinal bleeding and his post mortem examination had shown 'calcified veins'. It was likely that other more subtle changes of PXE had been missed.

Kat & Prick (1940), quoted by Carlborg (1944), mentioned a woman with PXE and thyroid disease, whose father, two brothers and one sister were also affected.

Marchionini & Turgut (1942), quoted by Berlyne, Bulmer & Platt (1961) and later by Wise (1966), described an affected mother and three daughters.

Carlborg (1944), in a detailed study of the peripheral vascular complications of the syndrome, described several interesting families which are described below.

Case 1. A woman aged 25 years had typical cutaneous PXE and angioid streaks of the retina. Although hypertensive when originally examined, this had improved on re-examination 5 years later. Her mother and sister had PXE.

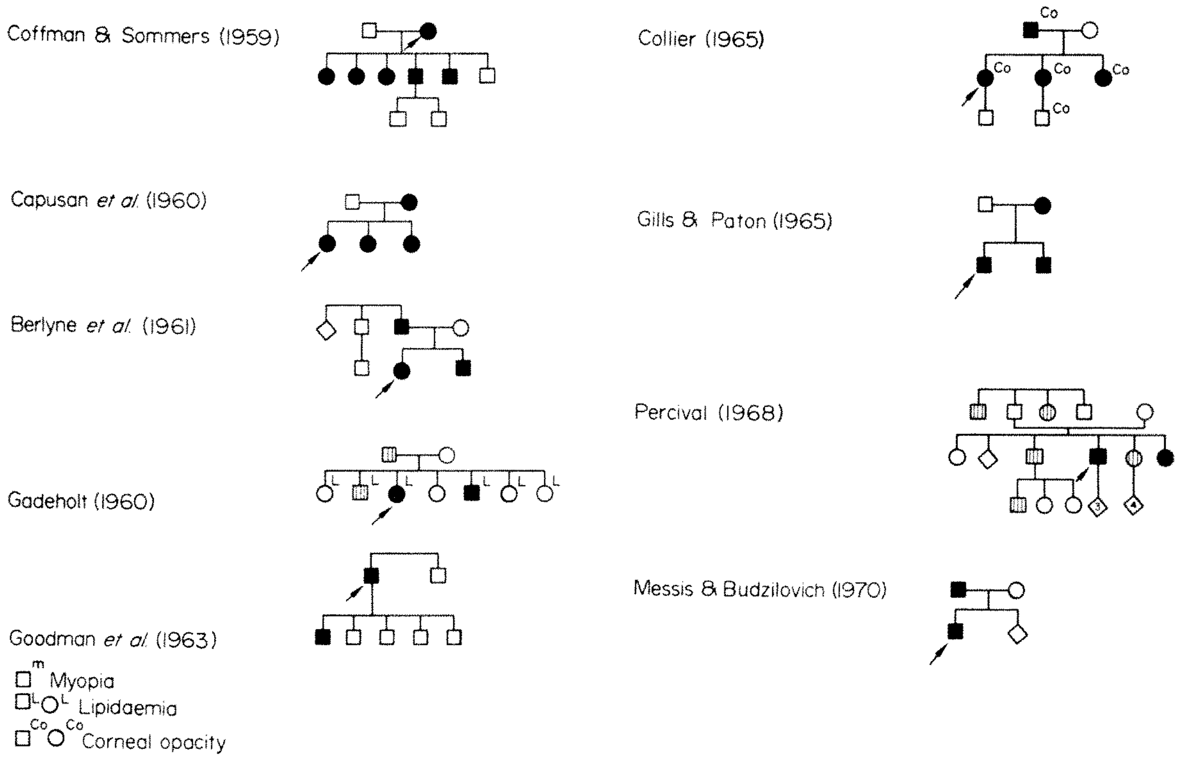

FIGURE 2. Pedigrees of possible autosomal dominant type I families reported.

Case 6, who was also Grönblad's (1929) third patient, had visual loss following an injury to her right eye. Two sisters had classical cutaneous PXE and one brother had similar changes. Her father had a 'weather-beaten complexion' which was probably extensive solar elastosis. This correlated with several other families in the literature.

Case 8, who was Grönblad's second patient, developed cutaneous PXE in 1929, followed 7 years later by central retinal disease. He had moderate hypertension with electrocardiographic changes of left ventricular hypertrophy, left bundle branch block, and cardiac ischaemia. His father had solar elastosis.

Applemans & Lebas (1953) described a 57-year-old Italian woman with extensive cutaneous PXE and a degenerative retinopathy. Her son had classical cutaneous and retinal disease whilst her daughter had the retinal changes alone.

Cahill (1957) described an autosomal dominant pedigree. The proposita, a 39-year-old woman, presented with impaired central vision and intermittent claudication. She had classical cutaneous PXE. Two of her four children were myopic and one had the typical cutaneous changes. Her brother had cutaneous PXE, visual impairment and optic atrophy as well as hypertension. Her mother had a 21-year history of visual impairment and had typical cutaneous PXE. The mother's brother and the uncle of the propositus had identical cutaneous and retinal changes.

Coffman & Sommers (1959) described an important pedigree. The proposita, a 60-year-old female, presented with congestive cardiac failure secondary to mitral stenosis. At post mortem examination she was found to have cutaneous and cardiac PXE. Three of her children had biopsy-proven cutaneous

PXE. They also had various cardiac abnormalities, which included aortic stenosis with incompetence, aortic stenosis and mitral stenosis. Two others had cutaneous PXE without any cardiac lesions. The valvular changes were reminiscent of the Marfan and Ehlers–Danlos syndromes (Beighton, 1970; McKusick, 1972).

Capusan *et al.* (1962) described a pedigree in which the proposita had PXE, Albers–Schönberg disease and malocclusion of the teeth. Her two sisters had cutaneous and ophthalmic PXE, and her mother had Albers–Schönberg disease, cutaneous and ophthalmic PXE.

Gadeholt (1960) described an unusual family. The proposita had PXE, hypercholesterolaemia, intermittent claudication and an increased basal metabolic rate. Two siblings had elastic degeneration of the skin, which was probably solar elastosis. The youngest sibling had hypercholesterolaemia and pituitary infantilism. The father of the proposita had died prematurely of coronary artery disease, which was presumed to have been secondary to the hypercholesterolaemia, but might have resulted from the PXE.

Berlyne *et al.* (1961) reported an 18-year-old girl with typical PXE, intermittent claudication and recurrent haematemeses. Her brother had angioid streaks, a degenerative retinopathy and diminished peripheral pulses, and her father had cutaneous and retinal PXE.

Goodman *et al.* (1963) described a 33-year-old man who had a 14-year history of indigestion, associated with several haematemeses. There was extensive cutaneous PXE of the neck, flexures and umbilicus. Gastroscopy showed a yellow mucosal infiltration. His son had typical cutaneous PXE.

Collier (1965) described a family in which the proposita, a woman aged 36 years, had presented with colloid degeneration of the cornea. She also had posterior synechiae and macular degeneration of the optic fundus. She had classical changes of cutaneous PXE. Her father and two sisters had similar punctiform corneal degeneration at the level of Descemet's membrane. Her son had normal fundi, but one of her nephews had similar corneal opacities.

Gills & Paton (1965) described a 12-year-old boy with cutaneous PXE and retinal pigmentation. His mother and maternal grandmother had similar cutaneous and ophthalmic changes.

Percival (1968) described a most important pedigree. The propositus presented at the age of 35 years with a 'brain stem syndrome' which was diagnosed as basilar insufficiency. He had classical cutaneous PXE and a severe degenerative retinopathy. Later he developed intermittent claudication, and was found to be hypercholesterolaemic. It was not clear if the PXE, hypercholesterolaemia or both were responsible for the premature vascular occlusive disease. A sister had classical cutaneous and ophthalmic PXE. One parent and several siblings apparently had solar elastosis. Percival interpreted this as a family with separate autosomal recessive PXE and autosomal dominant solar elastosis. An alternative possibility is that solar elastosis and cutaneous PXE were pleiotropic effects of the PXE gene.

(*b*) *Autosomal dominant type II group* (Figs 3 and 4)

This group is less distinctive than the last and could have been overlooked by clinicians seeking the more classical syndrome. Increased cutaneous extensibility, blue sclerae and a high arched palate were usual. The syndrome could be confused with some types of the Ehlers–Danlos syndrome, which would explain occasional case reports in which both diseases have affected the same patient.

Carlborg (1944) quoted the patient described by Schrader (1906) with extensive angioid streaks. The father and brother of this man had died prematurely of arterial disease and may have been affected.

Carlborg's third patient was a 31-year-old woman with mild cutaneous PXE. Visual impairment had followed an eye injury. She was also myopic. Her sister had typical cutaneous PXE and myopia. Both parents were reputedly normal, but increased cutaneous extensibility, prominent choroidal vessels, loose-jointedness and blue sclerae may have been overlooked.

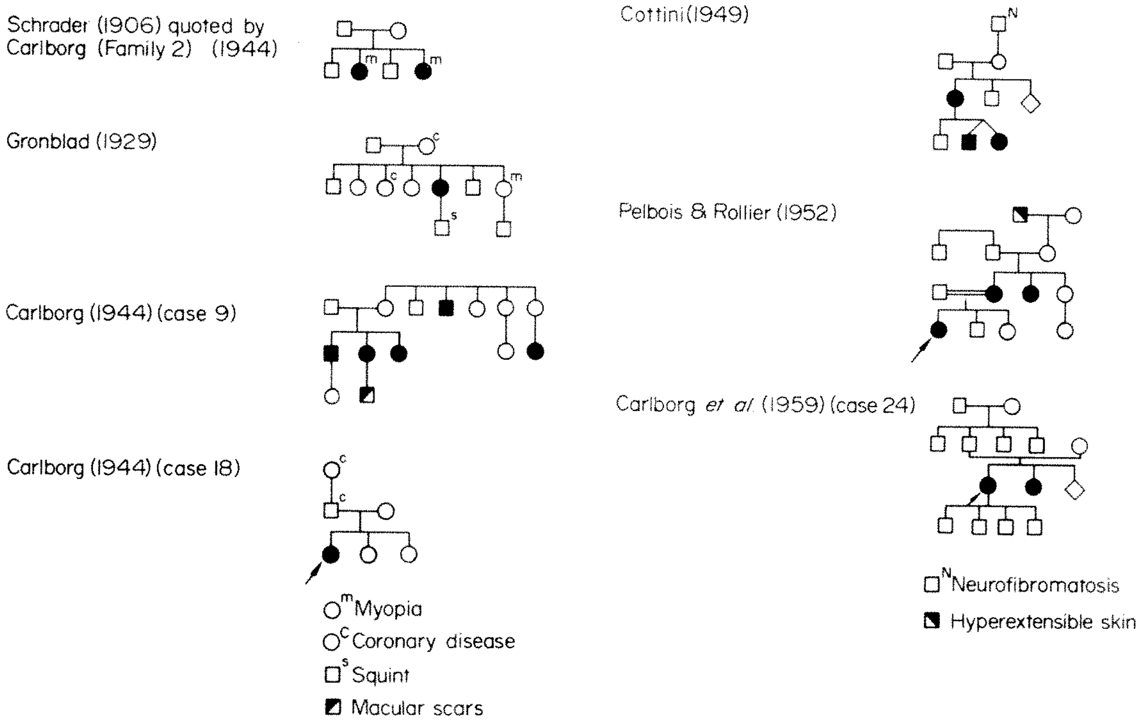

FIGURE 3. Pedigrees of possible autosomal dominant type II families reported.

Carlborg's case 9 was of particular interest. The proposita, a woman aged 48 years, had had a hysterectomy for recurrent menorrhagia. She had typical cutaneous PXE, a mild—2 dioptre—myopia and angioid streaking of the retina. Her elder brother died following a haematemesis and was known to have had angioid streaks. Post mortem examination showed that there was elastic degeneration of the aorta and the mitral valve. The haematemesis was secondary to cirrhosis of the liver and portal hypertension. His son had an unusual retinal pigmentation. Another sister of the proposita was described by Carlborg as case 13. She had a late onset myopia, with a mild disciform fundal degeneration and angioid streaks. There was typical cutaneous PXE over the neck.

Her son had a 'mottled' fundal pattern, typical of the dominant type II group. A second sister of the proposita had pigmented cutaneous lesions of the neck.

Cottini (1949) described an unusual patient with PXE, hyperextensible skin and premature canities. One of her three children had died prematurely and non-identical twins each had increased cutaneous extensibility and strabismus. One of the twins was myopic. Her maternal grandmother had von Recklinghausen's disease and increased cutaneous extensibility. Such clinical heterogeneity is suggestive of type II dominant disease.

Pelbois & Rollier (1952) described the apparent coincidence of PXE and Ehlers–Danlos syndrome in the same patient. The proposita, a woman aged 22 years, had cutaneous PXE of the neck, axillae and groins. She also had hypermobile joints and molluscoid pseudotumours. The fundi showed angioid streaks and 'pearly nodules', probably so-called salmon spots, typical of the dominant type PXE (Pope, 1974b). Her mother had hyperextensible joints, cutaneous laxity and premature ageing. Although Pelbois & Rollier (1952) considered that she did not have PXE, her sister, that is the maternal aunt of the proposita, had classical cutaneous changes. The fact that the father of the proposita

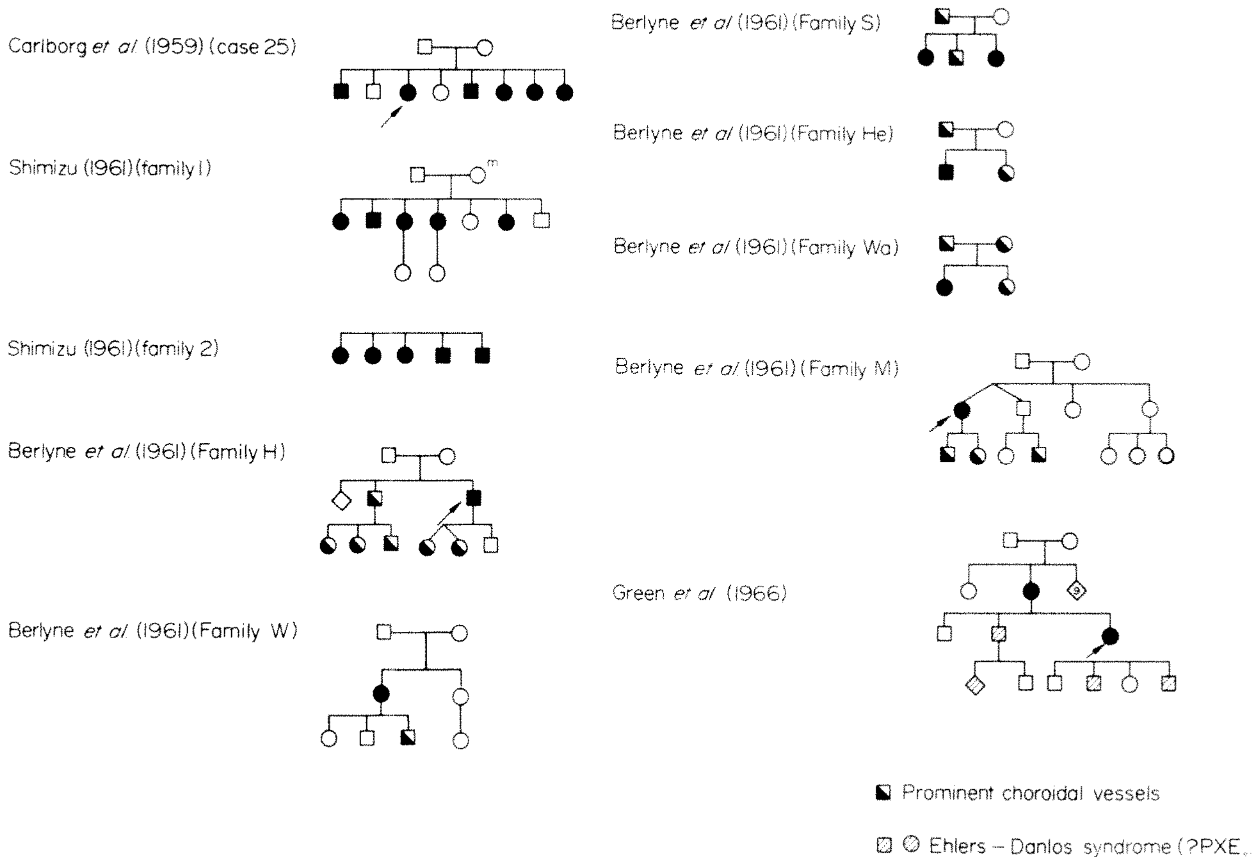
Carlborg et al (1959) (case 25)
Shimizu (1961) (family 1)
m
Shimizu (1961) (family 2)
Berlyne et al (1961) (Family H)
Berlyne et al (1961) (Family W)
Berlyne et al (1961) (Family S)
Berlyne et al (1961) (Family He)
Berlyne et al (1961) (Family Wa)
Berlyne et al (1961) (Family M)
Green et al (1966)
Prominent choroidal vessels
Ehlers – Danlos syndrome (?PXE

FIGURE 4. Pedigrees of possible autosomal dominant type II families reported.

was her mother's paternal cousin was irrelevant to the inheritance of the PXE, because the maternal grandfather of the proposita had hyperextensible skin and joints, which was therefore independent of the consanguineous relationship. The diverse clinical signs were typical of dominant type II PXE and previous case reports which described the Ehlers–Danlos syndrome and PXE in the same patient were really examples of this type.

Carlborg *et al.* (1959) described a second series of affected patients. Patient 18 had typical cutaneous PXE and was myopic. He had two normal sisters, but his parents had died and could not be currently reported. Patient 24 was tall and slenderly built, as were three of her four children. She had sparsely distributed cutaneous PXE with some very unusual palmar lesions. The fundi showed angioid streaks and macular degeneration. One sister had cutaneous PXE. The 'Marfanoid' habitus of the proposita and her four children probably indicated a generalized connective tissue defect.

Patient 25 was one of a remarkable pedigree, in which five of seven siblings had PXE. The proposita had a history of intermittent claudication. There was moderately severe cutaneous PXE and the fundi showed angioid streaks. The four affected siblings of the proposita were described by Carlborg as patients 26–29. One had claudication with angioid streaks, a third had cutaneous PXE and retinal degeneration, and a fourth had cutaneous and ophthalmic PXE. The mother of the proposita was normal.

Shimizu (1961) described two families with histories which strongly suggested autosomal dominant type II disease. The proposita of family 1, a woman aged 22 years, was myopic and had the typical yellow papules of cutaneous PXE over the neck, shoulders, umbilicus and axillae. Her fundi showed an irregularly mottled pattern, which slit-lamp examination showed lay deep to the retinal vessels. Two of her sisters had identical cutaneous and fundal changes, whilst an older brother and sister had the fundal mottling without any cutaneous changes. The mother and father of the proposita were normal.

Family 2 comprised a sibship of five in which the proposita, a 21-year-old female, had a 2-year history of a papular rash of the neck and axillae and diffusely mottled fundi. Her four siblings had similar fundal mottling, without any obvious cutaneous changes.

The two families were very similar to others with autosomal dominant type II PXE examined in Britain (Pope, 1973).

Berlyne *et al.* (1961) described at least five families in which prominent choroidal fundal vessels were associated with PXE. They regarded them as marking the heterozygous carriers of the homozygous recessive type of PXE. They did not mention other associated signs, such as increased cutaneous extensibility, loose-jointedness or myopia. In contrast, Goodman *et al.* (1963) were unable to confirm such prominent retinal vessels in any of their patients. This was possibly because they were of the 'classical' type and more likely to have dominant or recessive type I disease. Their case histories supported this hypothesis.

Berlyne *et al.* (1961) described several other families in their paper which were of great importance. The propositus of family 'H' had typical PXE, while his brother and sister had prominent choroidal vessels, without obvious cutaneous changes. The propositus of family 'W', a woman aged 44 years, had an 8-year history of cutaneous PXE. Her brother had prominent choroidal fundal vessels. The propositus of family 'W.A.' had a history of recurrent haematemeses and showed classical cutaneous PXE. Both parents and his sibling had prominent choroidal fundal vessels.

'S' had two affected sisters, one of whom had repeated haematemeses. Their father and brother reputedly had prominent choroidal fundal vessels, which suggested the autosomal dominant type II PXE.

Green, Friedman & Banfield (1966) described a patient said to have both Elders–Danlos syndrome and PXE. The proposita had increased cutaneous extensibility and a bruising tendency, as well as a retinopathy. Her mother, brother, and four of her children also bruised easily. There was no comment about myopia or prominent choroidal vessels.

(*c*) *Autosomal recessive families* (Figs 5–7)

Autosomal recessive traits are transmitted in such a way that the unaffected parents of affected individuals are obligatory unaffected heterozygotes. On average, one-quarter of the children of such marriages are affected, provided there is no bias of ascertainment. There were many reported families with an autosomal recessive pattern, although others may have been new dominant mutations. No examples of recessive type II PXE were found, and this account is entirely of type I families.

Chauffard's famous patient (1889), later redescribed and renamed as PXE by Darier (1896), first drew attention to the syndrome. There was no relevant family history.

Doyne (1889) was amongst the first to describe angioid streaks, followed closely by de Schweinitz (1897), who described the first affected family. The propositus, a 47-year-old man, had angioid streaks, haemorrhage and a degenerative retinopathy. Three years later, his 43-year-old brother developed very similar fundal changes. A third brother was affected but not described in detail. There was no comment concerning the parents and children of these men, but they were presumably unaffected.

Calcott Fox (1910), in a very short communication, recorded two affected sisters with classical cutaneous PXE.

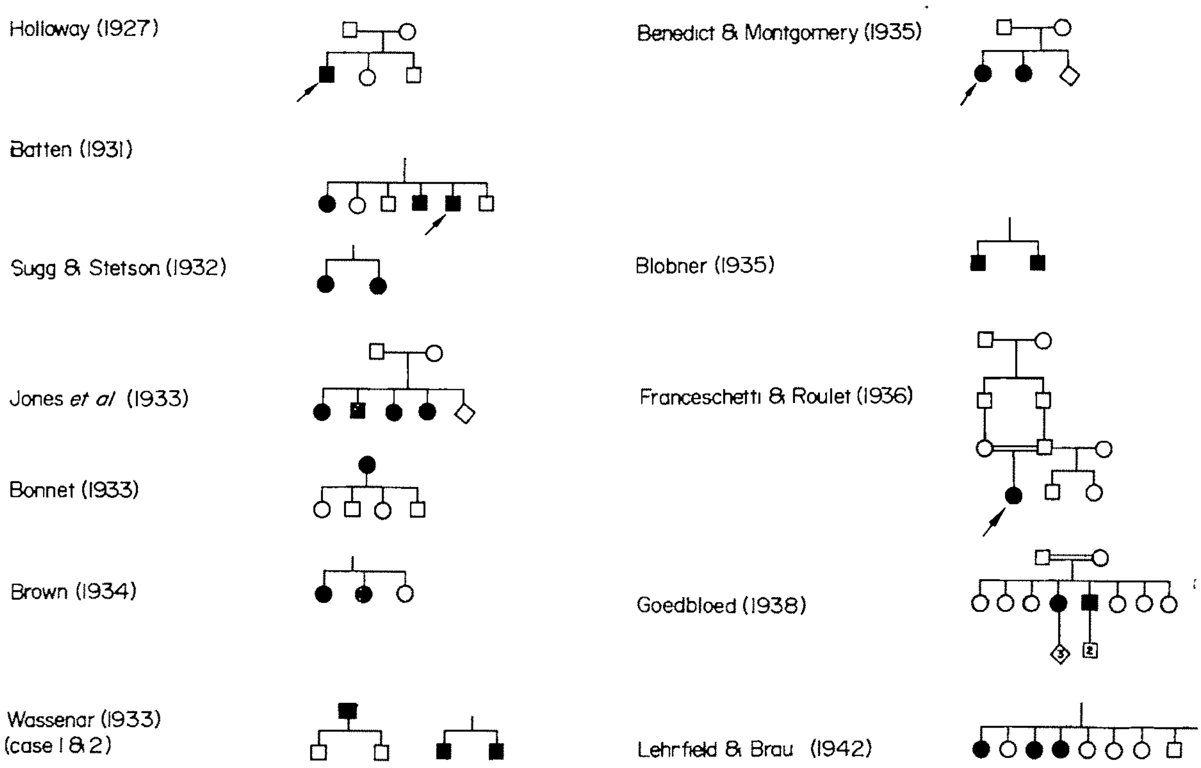

FIGURE 5. Pedigrees of autosomal recessive families reported.

Holmes Spicer (1919) recorded a brother and sister with angioid streaks, but did not comment upon their parents.

Throne & Goodman (1921) reviewed all the available literature from 1896 onwards, and mentioned Werthe's patient (1904). The proposita, a woman aged 28 years, had a history of PXE, weakness, anaemia, and recurrent indigestion. Here severe cutaneous disease was clearly shown in a set of stereoscopic pictures. Two sisters were affected but there were normal parents and other siblings.

Throne & Goodman (1921) described an interesting family, in which the proposita, a woman aged 39 years, had typical cutaneous PXE of the neck and axillae. Her younger sister was affected, but both parents were normal. Excellent paintings of the characteristic histological changes, as stained by haematoxylin and orcein, accompanied the article.

Kissmeyer & With (1922) described a woman with cutaneous PXE of the neck and flexures. Two of her siblings were affected, but her daughter and parents were not.

Milian & Lambert (1926) described a woman with PXE, whose father and one sibling had achondroplasia. There was no other affected family member with PXE.

Calhoun (1927) described a pedigree in which the propositus presented with sudden visual loss of his right eye, with choroiditis, angioid streaks and retinal haemorrhage. One brother had choroiditis and another a history of recurrent haematemeses. Other siblings had died of apparently unrelated disorders, such as cerebral glioma, thyrotoxicosis, eclampsia, and coronary occulusion respectively. Calhoun hinted that there were other family members with angioid streaks, but did not clarify this. He was very near the truth in postulating that angioid streaks and choroiditis were related to the

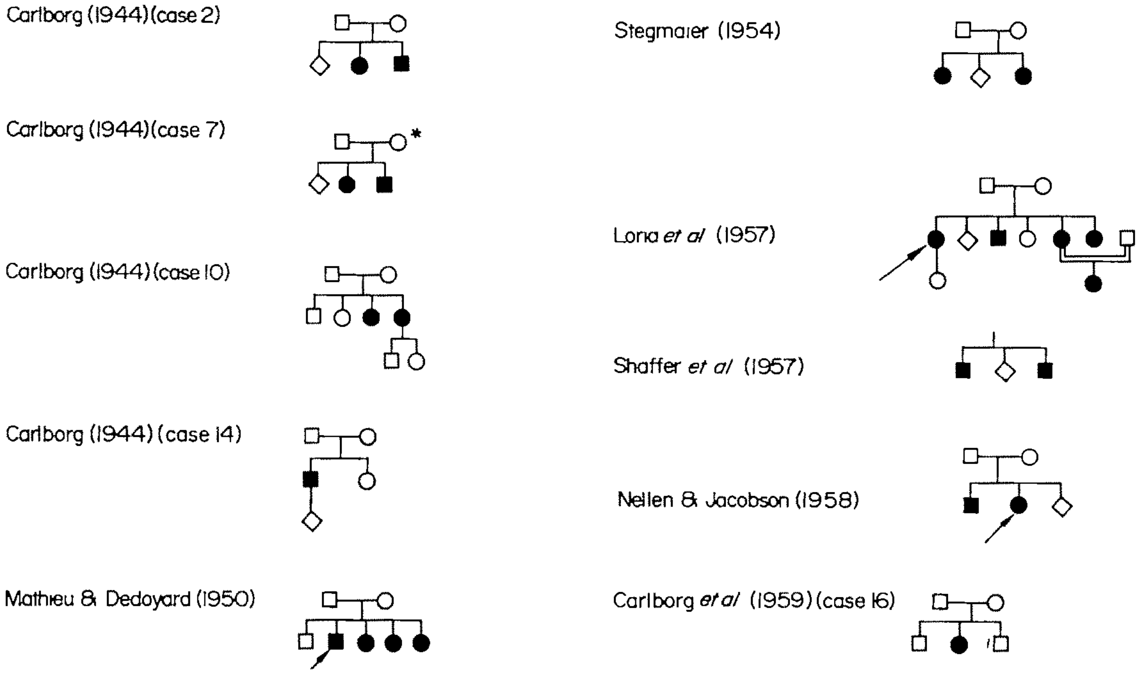

FIGURE 6. Pedigrees of autosomal recessive families reported.

intestinal haemorrhage and coronary artery occlusion, the common factor being a disorder of the small blood vessels.

Holloway (1927) described a man with a degenerative retinopathy. His two siblings were unaffected, although his parents had died of a cerebral haemorrhage and coronary artery disease respectively.

Batten (1931) conclusively established an association between angioid streaks and choroidal degeneration. He published some of the best fundal paintings ever made of retinal PXE and traced the progression from an initial haemorrhagic phase to the final stage with scarring, pigmentation and degeneration. One of his sibships had three affected and three normal individuals. All those affected had both a degenerative retinopathy and angioid streaks.

Then Grönblad (1932) published her famous monograph, which unified the separate dermatological and ophthalmic syndromes into a single disorder. The first of her three patients had clinical features of the dominant type II group, while the others were certainly recessive type I.

Sugg & Stetson (1932) reported two diabetic sisters with cutaneous and ophthalmic PXE. The 46-year-old proposita had bilateral angioid streaks and a choroiditis of the right fundus. Her sister had cutaneous PXE and bilateral angioid streaks.

Jones *et al.* (1933) described several patients, some of whom have already been discussed above. Patient 4 had a history of scarred skin around the neck since childhood, angioid streaks and a degenerative retinopathy. Two of his sisters had cutaneous, but no ophthalmic PXE. There was no comment concerning the parents.

Brown (1934) described a family with two out of three siblings affected, but no affected children or parents.

Benedict & Montgomery (1935) reported two affected siblings. The proposita had a history of cutaneous PXE since the age of 12 years and also had increased serum lipids and fatty acids. There was a similarly affected sister.

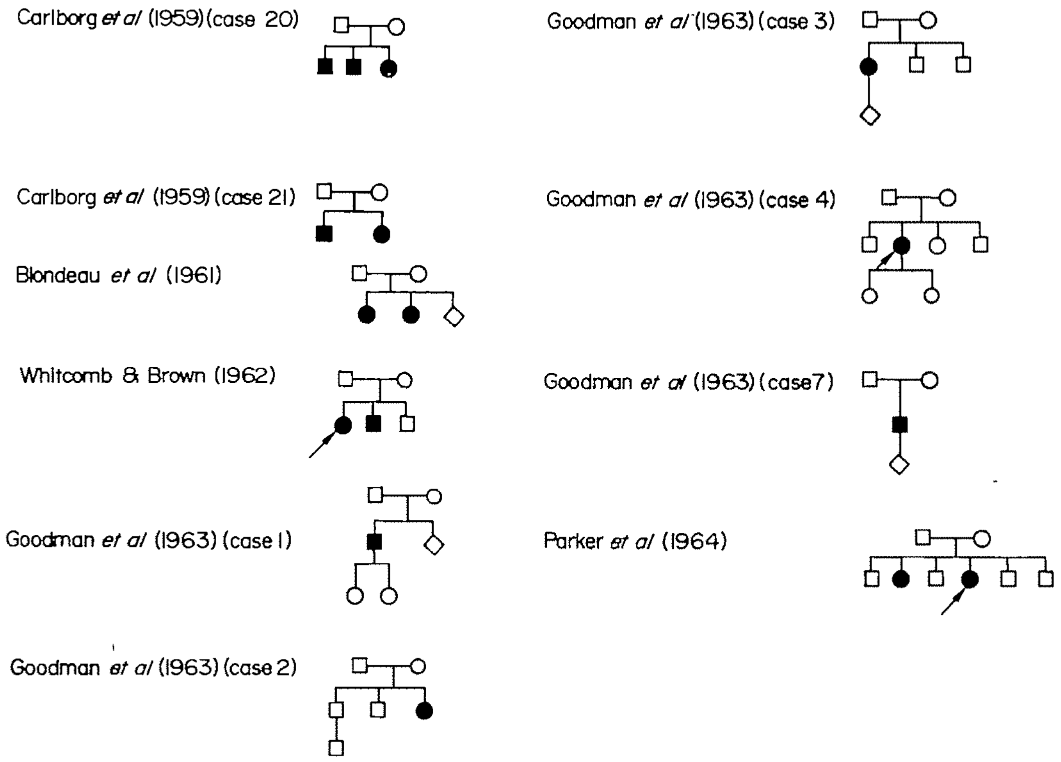
Carlborg *et al* (1959)(case 20)
Carlborg *et al* (1959) (case 21)
Blondeau *et al* (1961)
Whitcomb & Brown (1962)
Goodman *et al* (1963) (case 1)
Goodman *et al* (1963) (case 2)
Goodman *et al* (1963) (case 3)
Goodman *et al* (1963) (case 4)
Goodman *et al* (1963) (case7)
Parker *et al* (1964)

FIGURE 7. Pedigrees of autosomal recessive families reported.

Franceschetti & Roulet (1936) reported a 54-year-old woman with some recent visual deterioration. Her right fundus showed angioid streaks and macular pigmentation and the left contained macular haemorrhages. She was the illegitimate daughter of paternal first cousins, but her father had subsequently sired normal children, which was strong circumstantial evidence of autosomal recessive inheritance.

Bonnet (1933) described a 37-year-old woman with angioid streaks and macular scarring. She had four normal children, although no comment was made about her parents.

Wassenar (1933) described two patients with PXE. The first, a man aged 38 years, had angioid streaks and a choroiditis. Both his sons were unaffected. The second, a man aged 56 years, presented with a central scotoma from a macular exudate. His brother was similarly affected, but his parents and children were not commented upon.

Blobner (1935) described two affected brothers with cutaneous and ophthalmic PXE.

Goedbloed (1938) described a 46-year-old man with poor vision of the left eye which contained angioid streaks and haemorrhages. The right eye showed angioid streaks alone. Although his sister had cutaneous PXE, there were four other normal siblings and unaffected but consanguineous parents.

Lehrfield & Brau (1942) described a pedigree of three affected females out of eight siblings. Two of the three had classical cutaneous PXE and all had a degenerative retinopathy.

Touraine (1940) quoted evidence in support of both autosomal dominant and recessive inheritance, but unfortunately did not publish any pedigrees. He examined data from 102 families and considered twenty-nine to have been recessive compared with only five dominant.

Carlborg's series (1944) was the most comprehensive to that date and contained examples of both autosomal dominant and recessive inheritance. Patient 2, a female aged 20 years, had bilateral angioid streaks which surrounded the optic disc. There was cutaneous PXE of the neck as well as infiltration of the buccal mucosa. A severe haematemesis had followed an attack of epigastric pain. A younger sister had cutaneous PXE, but the parents and other siblings were normal. Patient 7 was a woman aged 42 years with a history of cutaneous PXE since childhood. The visual acuity was impaired with disciform macular degeneration and typical angioid streaks of the retina. A younger brother had angioid streaks and macular pigmentation. Both parents were unaffected. Patient 10, a woman aged 40 years, had developed cutaneous PXE 14 years earlier, with extensive involvement of the neck, axillae, flexures and, rather unusually, the breasts. A younger sister had angioid streaks. Two other siblings were unaffected. Patient 14, a woman aged 52 years, had a 4-year history of defective vision. She had bilateral choroiditis and her parents' other eight children were unaffected.

Mathieu & Dedoyard (1950) described a sibship of five individuals in which the propositus, a man aged 44 years, presented with premature ageing and PXE. His three sisters were also affected, but none was formally examined. Both parents and the brother of the propositus were unaffected.

Bafverstedt & Lund (1955) described a 9-year-old child with a history of intermittent claudication, with cutaneous PXE of the neck, arms, legs, trunk and axillary folds, but normal fundi. The parents and only sibling of the propositus were normal.

Carlborg (1959) described a second series of families, three of which had histories compatible with autosomal recessive PXE. Patient 16 was a woman with a history of cutaneous PXE since the age of 4 years. Haemorrhage of the right fundus had followed an injury. There were unusually extensive cutaneous changes, which may have been the very rare recessive type II variant. Her siblings and parents were unaffected. Patient 17 had cutaneous PXE. There were no other affected family members.

Loria *et al.* (1957) described a 49-year-old female with typical cutaneous PXE. She also had a right sided nephrolithiasis and she had had a hysterectomy for menorrhagia. She had injured her right eye which showed a choroiditis. Three of her many siblings had cutaneous PXE, and there was also an affected nephew, the son of consanguineous parents. The parents of the proposita were unaffected.

Shaffer, Copelman & Beerman (1957) described a sibship with three affected males. There was no comment about the remaining siblings and parents.

Nellen & Jacobson (1958) described a 31-year-old female with a 15-year history of recurrent haematemeses. An older brother had died aged 34 years of coronary artery disease and the propositus showed the very unusual sign of coronary artery calcification clearly visible on chest X-ray. This implied that her coronary vessels had undergone elastic degeneration followed by calcification, and that a similar process had contributed to her brother's premature death.

Blondeau *et al.* (1961) described a patient with typical cutaneous PXE and angioid streaks. Only one of her three siblings was affected.

Whitecomb & Brown (1962) described a 22-year-old female with a history of recurrent gastrointestinal haemorrhages and anaemia. She had typical cutaneous PXE. One sibling also had similar cutaneous changes, whilst the other, and both parents, were normal.

Goodman *et al.* (1963) described twelve affected families. Family 5 has been included as an example of autosomal dominant PXE. Families 1, 2, 3, 4, and 7 had single affected individuals without any detailed family data. Some were probably examples of autosomal dominant disease. Family 6 comprised seven siblings, of which no less than four boys and two girls had PXE. None had affected children or parents.

Remacle (1964) described a patient aged 64 years, with hypertension, angioid streaks and impaired peripheral pulses. A 70-year-old brother also had cutaneous PXE of the neck and his fundi showed retinal degeneration and choroidal sclerosis. Their parents and other siblings were normal.

Parker *et al.* (1964) described two sisters with PXE and hypertension. The elder presented aged 9 years with an unexplained progressive malignant hypertension, fundal haemorrhages, exudates, and papilloedema. Despite intensive investigation and an adrenalectomy in an attempt to control the hypertension, the patient died. Later, her younger sister also became hypertensive and was incidentally noted to have classical cutaneous PXE. This was previously unsuspected in her elder sibling but re-examination of post mortem material showed typical arterial medial elastic degeneration. Their parents and three male siblings were unaffected.

Consanguinity

In this review, consanguinity was mentioned in only four families; Franceschetti and Roulet (1936), Goedblad (1938), Pelbois (1952) and Laurier and Kennedy (1957). Wise (1966) mentioned nineteen affected families with evidence of consanguinity. He considered that the consanguinity rate among affected patients was at least 9% higher than the reported rate in North America and Europe.

GENETIC ANALYSIS OF THE DATA

Clinical characteristics

The clinical characteristics of the various selected families have been summarized in Table 1b. Allocation to one or other of the groups depended solely upon the clinical characteristics of those affected. Those considered autosomal dominant on clinical grounds generally turned out to have several affected generations, although naturally, occasional sporadic examples were found. The patterns of parent–child transmission confirmed this impression. Similarly, those individuals classified as autosomal recessive on clinical grounds had affected siblings and children but not affected parents. These were just the patterns which would have been expected for autosomal recessive inheritance. The clinical characteristics which determined the allocation to one or other of the groups have been already discussed in the opening section of this paper.

Results (Table 1)

There were 129 patients with sufficient clinical data for analysis, of whom forty-one were considered autosomal dominant type I, thirty-two dominant type II, and fifty-six autosomal recessive type I. Only one was classified as recessive type II. In general, the differences between the groups resembled those described by Pope (1973, 1974a, b) in the British series and summarized in Table 1a. They have been discussed under the headings of cutaneous, vascular, ophthalmic, and miscellaneous complications.

(1) *Cutaneous complications.* The dominant type I group almost invariably (99%) showed a classical cutaneous pattern compared with 33% of the dominant type II, and 75% of the recessive group. Similarly, the dominant type II group often had either a very mild cutaneous change (the so-called macular pattern) or no change at all. The frequency was 60% compared with only 5% of the dominant type I and 1·8% of the recessive group. Similarly, a generalized increase of cutaneous elasticity was confined to the dominant type II group, with 15% affected. Only one patient had cutaneous change of sufficient severity to have been considered a recessive type II variant.

(2) *Vascular complications.* Vascular complications, which included intermittent claudication, coronary artery occlusion and cerebrovascular disease, were commonest among the dominant type I group, just as in the British survey. Thus 30% of the dominant type I group had one or other vascular complication, compared with none of the type II and only 3·5% of the recessive groups. Intermittent

claudication affected 12% of the dominant type I group compared with 5·2% of the recessive, though cerebrovascular complications were rare in both. Hypertension was also uncommon, and affected 12% of the dominant type II group. Haematemeses, which were such a common feature of the recent (1970) survey, and which favoured the recessive group, were uncommon and evenly distributed, with dominant type I, 7·5%; dominant type II, 6%; and the recessive, 5·2%.

(3) *Ophthalmic complications*. The same striking differences that characterized the British survey (Pope, 1973) were evident here. Thus, the dominant type I group most commonly showed a severe degenerative retinopathy (62·5%) and angioid streaks were comparatively rare (10%). By contrast, the dominant type II retinae were very characteristic and rarely showed choroidoretinitis (3%). They commonly showed the so-called peau d'orange pattern (30%) or prominent choroidal vessels (12%). Myopia affected 21% of this group. The recessive group I was somewhat intermediate to the two dominant, except that angioid streaks were somewhat commoner.

Analysis of the pooled genetic data

The pedigree data were analysed in two ways. The first was by pedigree analysis of each individual family and has already been discussed. The second method utilized the pooled data to compare the numbers of affected and unaffected individuals within each group. Bernstein's (1929) method was used to test directly the Mendelian hypothesis that such ratios should have been one to one for the dominant group and one to three for the recessive group. The analyses showed a goodness of fit for the autosomal dominant type II type. The details have not been included in this paper, but can be made available by the author.

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An evaluation of Na^+, Cl^- and pH ion-specific electrodes in the study of the electrolyte contents of epidermal transudate and sweat

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SUMMARY

Ion-specific electrode detectors have been used to measure electrolytes in various biological fluids, including sweat.

As part of a study of skin barrier function, we have used such electrodes to detect the presence of sodium, chloride and hydrogen (pH) in the transepidermal water loss.

This paper describes the effects of cellophane tape stripping (of human forearm epidermis *in vivo*) on surface electrolyte concentrations, in parallel with observations on transepidermal water loss and galvanic skin resistance.

Various methods are available for measuring accurately the rate of water loss from the skin surface, but previously available techniques for measuring skin surface electrolytes at the same time are difficult or inaccurate.

In cystic fibrosis of the pancreas, direct reading ion-selective electrodes have been used for measuring the electrolyte content of sweat. These electrodes are placed on the skin and the sodium (Na^+) and chloride (Cl^-) content of sweat are recorded directly on a meter (Kopito & Shwachman, 1969). A similar direct reading hydrogen-ion selective electrode has been used for measuring skin pH (Behrendt & Green, 1971).

A simple method of measuring electrolytes in epidermal transudate or sweat is needed. The existing methods use filter papers placed on the skin; necessarily large areas of the skin have to be covered to collect adequate amounts of epidermal transudate for analysis. Microscopic methods for sweat collection are difficult.

In contrast, the area of skin contact with direct reading ion-selective electrodes is very small, allowing measurement of electrolyte loss from small lesions or from small areas, experimentally exposed to solvents, or damaged by stripping, etc.

Using Na^+ and Cl^- electrodes placed directly on the non-sweating undamaged skin, we found the salt content was too low in epidermal transudate to allow accurate measurement, but reliable pH readings were obtained. Sweating was inhibited by the topical application of poldine methosulphate.

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When skin was stripped, using cellophane tape, the electrolyte concentration of the epidermal transudate increased and was measured by the ion-selective electrodes. These data were compared with other parameters of barrier function, namely transepidermal water loss and galvanic skin resistance.

The electrolytes in epidermal transudate in psoriasis and eczema and in sweat have been measured by the ion-selective electrodes and the data compared with those obtained by using the conventional filter paper method.

MATERIALS AND METHODS

In order to measure trans-epidermal water loss due to diffusion (TEWL) eccrine sweating must be absent. Sweating was inhibited by the local application of 4% poldine methosulphate under polythene occlusion to a 4 cm^2 area of forearm skin in normal volunteers. The following day the absence of sweating was confirmed by measuring the treated skin's galvanic resistance. The following parameters were then measured:

(A) Transepidermal water loss (TEWL) which was then corrected for skin temperature (Grice *et al.*, 1971). Dry nitrogen was passed through a capsule covering a 10 mm^2 area of skin. The resulting humidity was measured electrolytically. The capsule was made of polytetrafluoroethylene and was an adaptation of that used by Spruit (1972).

(B) Na^+ and Cl^- and pH were measured rapidly on the skin surface by means of the flat-headed direct reading ion-selective electrodes.

(1) Na^+ combination electrode (Orion, Model 96-11).
(2) Cl^- combination electrode (Orion, Model 96-17-00).
(3) H^+ selective electrode for measuring skin pH (Electronic Instruments (EIL) Flat head electrode 33, 1070, 230).

The dry electrodes were placed in turn on the skin at the site where the TEWL had been previously measured. The skin surface electrolytes were read directly on the specific-ion meter (Orion 404).

(C) Galvanic skin resistance was measured by a resistance meter. A thin platinum U shaped wire loop (3 mm diameter) made contact with the skin. In order to complete the circuit through the body, an E.C.G. limb electrode coated with saline jelly was attached to the opposite arm. The resistance between the two electrodes was, therefore, essentially the skin resistance in contact with the platinum electrode (Bettley & Grice, 1965).

(D) Skin temperature was measured by a multi-channelled skin thermistor (Light Laboratories, Brighton).

Experimental procedures

Epidermal barrier function and stratum corneum stripping

(1) The anterior surface of the forearm was used in six normal volunteers. Sweating was inhibited as described above, and next day the various parameters (TEWL, Na^+, Cl^-, pH, galvanic skin resistance) were measured from an area of 1 cm^2 of skin. This area of skin was then 'stripped' using cellophane tape. After every 2–4 strips, the various parameters were remeasured. Stripping was continued until the tape no longer adhered to the skin. In some subjects the rate of recovery of the barrier was then remeasured on the days following stripping.

(2) The anterior surface of the forearm was used in eight normal volunteers; a variation of the previous method was used while the TEWL was low, i.e. at the initial stages of the experiment. Green, Behrendt & Libien (1972) suggested that, when the skin was dry and the electrical contact

between skin and electrode was poor, the base of the electrode could be dampened with distilled water to form a water bridge. The readings thus obtained they suggested were accurate. We dampened the base of the Na^+ and Cl^- electrodes with distilled water. They said that little was known about a suitable salt bridge for Na^+ and Cl^- electrodes that assured good conduction without altering 'natural activities or sensitivity of the electrodes'. To avoid producing false results from the damp skin, the other parameters were measured on the skin adjacent to the Na^+ and Cl^- tested area.

Comparison of ion-selective electrodes and standard filter-paper technique

The results obtained with the direct reading ion-electrodes were compared with those derived by the standard filter-paper technique of collecting epidermal transudate or sweat. Two methods were used.

(a) *Sweating* was induced in ten subjects by sitting the subjects with their legs in a warm water bath. The water level came to the knees. The water temperature was maintained at 42°C and the room temperature at 30°C.

The dry ion-selective electrodes were placed in turn in the liquid droplets of sweat, and Na^+, Cl^- and pH measured directly. These measurements were done at the same time as sweat was being collected by filter papers from adjacent skin areas. 540 Whatman filter papers were used in the sweat capsule fixed to the skin as described by Schwartz & Thaysen (1956) and Cage & Dobson (1965). Na^+ and K^+ in the filter papers were measured by flame photometry.

(b) *Psoriasis.* On psoriatic plaques, water and electrolytes were collected in filter papers under polythene occlusion as described previously (Grice, Sattar & Baker, 1973). Sixteen subjects were tested. On the same area of psoriasis, the Na^+ and Cl^- were measured on the skin directly, by placing dry electrodes on the skin. The electrodes were then dampened with distilled water and then replaced on the same area of psoriasis.

To ensure that scales contributed a negligible amount to the gain in weight of the filter paper, the following procedure was adopted in four cases. The weighed filter paper before use was allowed to equilibriate in a dessicator until constant weight was achieved. The dry weight (A) was noted and the filter paper allowed once more to equilibriate in room humidity. After being used on the psoriatic skin, the papers were re-weighed directly, then again after being dried (B). The difference between the two dry weights (B − A) of the filter paper was an approximate measure of the amount of scale removed.

RESULTS AND DISCUSSION

Epidermal barrier function and stratum corneum stripping

(1) Using direct reading electrodes on fourteen subjects, the mean Na^+ content of the epidermal transudate before stripping was 1·7 mEq/l (s.d ±0·7). After maximum stripping it rose to 126 mEq/l (s.d. ±31·5). The Cl^- content of the epidermal transudate was 2·8 mEq/l (s.d. ±3·5) before stripping and after maximal stripping rose to 102 mEq/l (s.d. ±11·0). The pH rose from 4·6 to 6·6. The skin galvanic resistance reflected these changes, the resistance falling from $1000 \times 10^6 \Omega$ to $1 \times 10^6 \Omega$. After maximum stripping, therefore, the electrolytes on the skin surface rose from a very low concentration and reached the concentration found in extracellular fluid. These findings are summarized in Table 1.

The transepidermal water loss increased about sixty-fold after maximum stripping. A similar increase of TEWL has been found by previous authors who suggested that the water loss from stripped skin is approximately the same as that from an aqueous protein solution (Monash & Blank, 1958; Spruit, 1970). The TEWL readings were probably abnormally high as the capsule was extremely small. (In the present series of tests TEWL was only used, however, for comparison purposes.)

TABLE 1. Effect of stripping stratum corneum with cellophane tape in fourteen subjects

	Before (mean, s.d.)	After (mean, s.d.)
TEWL $mg/cm^2/h$	1·2±0·8	60·8±29 3
Na^+ mEq/l	1·7±0·7*	126±31·5
Cl^- mEq/l	2·8±3 5*	102±11·1
pH	4·6±0.8	6·6±0 3
Skin resistance (Ω)	1000×10^6	1×10^6

* Water bridge.

An increased air speed through the narrow capsule inlets could account for high TEWL readings. Spruit (1972) showed that capsules enclosing 0·1 mm^2 skin areas gave falsely high results, but larger capsules (1 mm^2) were said to be accurate. The capsule we used enclosed a skin area of 10 mm^2.

(2) The results of stripping the skin are shown in Figs. 1 and 2. Dry Na^+ and Cl^- electrodes,

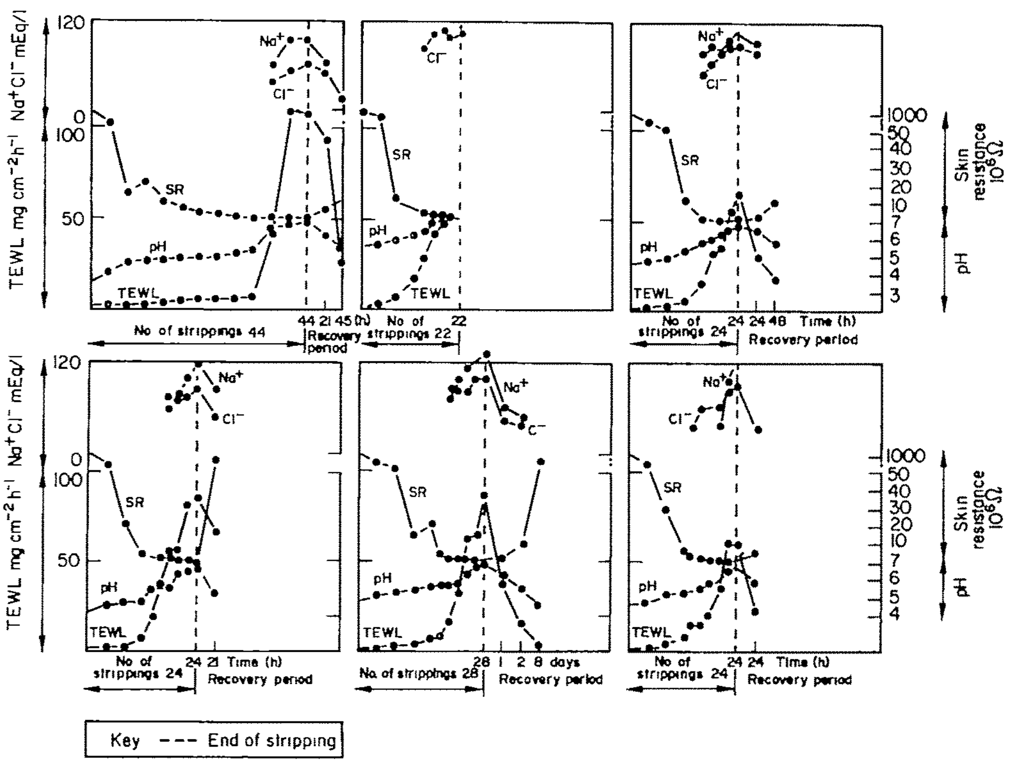

FIGURE 1. Removal of stratum corneum by stripping forearm skin in six normal subjects. Effect on transepidermal water loss (TEWL) Na^+, Cl^-, pH and galvanic skin resistance (SR).

when used on the non-sweating skin in six subjects in Fig. 1, did not produce enough electrical contact for accurate readings. Only on approaching maximum stripping were readings satisfactory.

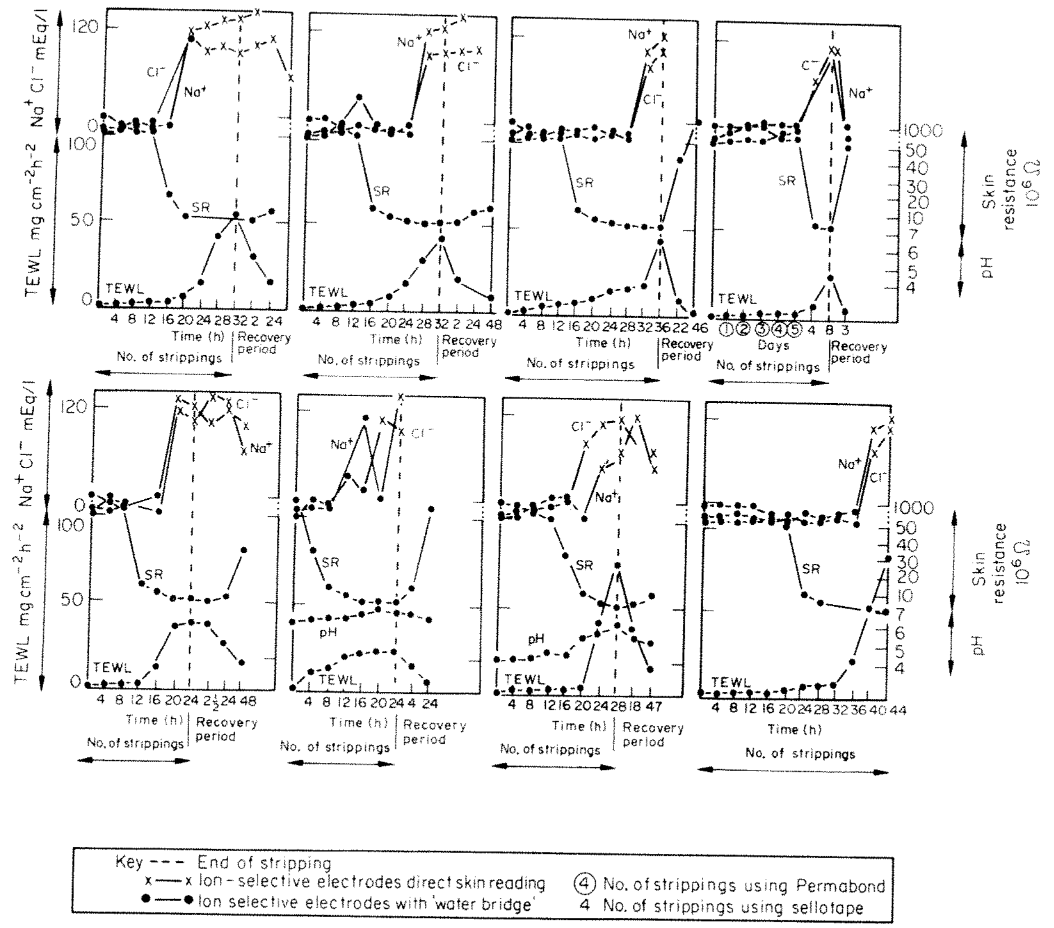

FIGURE 2. Removal of stratum corneum by stripping forearm skin in eight normal subjects using a 'Water bridge' for Na$^+$ and Cl$^-$ electrodes while the skin was dry.

Fig. 2 shows the use of the 'water bridge' for obtaining better skin contact when using the Na$^+$ and Cl$^-$ electrodes on dry skin (i.e. the bases of the electrodes were dampened by distilled water). Dry electrodes were used when approaching maximum stripping.

Figs. 1 and 2 both show that TEWL rose sharply as maximum stripping was approached.

The pH readings were satisfactory throughout on dry non-sweating skin with an intact barrier as well as when the barrier was destroyed, immediate consistent readings being obtained at all times.

Throughout the stripping period, the pH rose with the TEWL. The skin resistance seemed to follow inversely the pH pattern and started falling before the marked rise in TEWL. This would be as expected; that the skin resistance reflects the salt content of epidermal transudate and not the amount of water loss.

The Na$^+$ and Cl$^-$ concentration in the transudate reached extra-cellular fluid levels sharply, as maximum stripping was approached and at about the same time as the TEWL's sharp rise. This suggests that the barrier to the passage of Na$^+$ and Cl$^-$ and water through the skin was damaged *pari passu* by stripping. Hojyo-Thomoko & Kligman (1972) showed that cellophane tape stripping removed about two-thirds of the horny layer. After maximum skin stripping they found they could still produce a blister with cantharidin. They suggested that these few layers of stratum corneum remaining after maximum stripping were so loosened and damaged that they no longer functioned as a water barrier.

As the epidermis recovered, so all the parameters returned to more normal levels. Three to four days after stripping the water barrier function had nearly returned to normal. This is similar to the findings of others (Spruit & Malten, 1966; Eriksson & Lamke, 1971).

Ethyl cyanoacrylate dental adhesive (Permabond white) has been used successfully for stripping-off layers of stratum corneum (Marks & Dawber, 1971). In one of our experiments, Permabond was used initially, instead of cellophane tape, for stripping (Fig. 2, subject 4). It was found that after five successive applications of Permabond, the adhesive no longer adhered to the skin, and the deeper layers of the stratum corneum had to be removed with cellophane tape.

Comparison of ion selective electrodes and standard filter-paper technique

Sweating. The sweat electrolytes were measured at the same time from adjacent skin areas by the electrodes and by the sweat capsule containing the filter paper.

Na^+, Cl^- and pH electrodes were extremely easy to use and gave consistent results in the presence of visible sweating. As seen in Table 2, the level of Na^+ in sweat as measured by the filter paper method was similar to that found using the ion-selective electrode (correlation coefficient $f = 0{\cdot}8440$).

TABLE 2. Electrolytes in sweat; comparison of sodium levels by two methods—filter paper and ion-selective electrode

Electrolyte	Filter paper (mean, s.d.)	Ion-selective electrode (mean, s.d.)
Na^+ mEq/l	63·1 ± 18·3	56·6 ± 24·9
K^+ mEq/l	9.3 ± 3.7	—
Cl^- mEq/l		66 5 ± 16·0

Under conditions of minimal sweating, the pH electrodes were satisfactory but Na^+ and Cl^- electrodes needed the 'water bridge' to produce satisfactory contact. In view of the possible dilution factor, it was then difficult to know how much reliance to place on the reading obtained under conditions of minimal sweating. The pH readings for dry skin were immediately obtained and were consistent.

TABLE 3. Sodium (mEq/l) in epidermal transudate in psoriasis, eczema and normal skin; comparison of two methods—filter paper and ion-selective electrode

Method	Psoriasis (mean, s.d.)	Eczema (mean, s.d.)	Normal (mean, s.d.)
Filter paper	10·0 ± 5.6	12.2 ± 3·6	4·9 ± 2·8
Ion-selective electrode	11 9 ± 9·7	15·0 ± 11·6	9·0 ± 1·4
Number of subjects	15	4	3

Psoriasis and eczema. Table 3 shows that the mean sodium levels on the non-sweating plaques of psoriasis, eczema and normal skin were very similar, using the two different methods for collection. There was no statistical correlation (correlation coefficient f = 0·2520).

The amount of scale collected by the filter paper method in psoriasis was negligible. On four subjects, the scale collected on the filter paper during 4 h was 0–0·2 mg.

CONCLUSION

Although the water barrier of human skin has been extensively investigated, little interest has been taken in the barrier to electrolytes. A previous study (Grice *et al.*, 1973) showed that in active psoriasis the salt loss from the skin was extremely low in spite of a large water loss from the skin caused by impairment of the epidermal water barrier. This suggested a preservation of the electrolyte barrier in psoriasis in spite of damage to the water barrier.

The present investigation was undertaken to find a new method for investigating the electrolyte epidermal barrier. In our investigation the use of Na^+, Cl^- and H^+ ion-sensitive electrodes has enabled small areas of skin to be traumatized and investigated. H^+ sensitive electrodes have been used successfully previously for measuring skin pH (Behrendt & Green, 1971) as have Na^+ and Cl^- sensitive electrodes for sweat analysis (Hansen *et al.*, 1968).

The electrodes are simple and quick to use, a reading being obtained on a meter simply by placing the flat headed electrode in contact with the skin. The method should be useful for investigating skin barrier function as well as sweating.

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The effect of the activity of psoriasis on the response to autologous ground skin extract

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SUMMARY

The effects of autologous extracts of involved and macroscopically uninvolved psoriatic skin on epidermal DNA synthesis have been studied. Previous reports of inhibition of DNA synthesis were confirmed but in a number of patients a stimulant, rather than an inhibitory effect, was observed. The stimulant effect was only present when the disease was in an acute, eruptive phase and was more marked in the involved than in the macroscopically uninvolved skin.

Skin extracts have been shown to inhibit epidermal cell production (Bullough & Laurence, 1964; Marks, Fukui & Halprin, 1971). A stimulant effect of guinea-pig extracts on epidermal DNA synthesis has been reported by Hell (1970). Psoriasis is characterized by increased epidermal cell production and in this paper we report the effects of psoriatic skin homogenates on DNA synthesis in both involved and macroscopically uninvolved psoriatic epidermis. In view of the possibility that the effects of skin extracts might be influenced by the degree of activity of the psoriasis, attempts were made to assess the activity of the disease in each patient on the basis of clinical criteria and estimation of the erythema threshold to ultraviolet radiation (Hodgson & Hell, 1972).

METHODS

Thirty-four hospitalized patients (22 males and 12 females), with ages ranging from 22 to 69 years, gave their informed consent to participate in this study. All patients were in good general health and none was taking any drugs except for hypnotics and occasionally anti-histamines. The psoriasis affected from 10% to 90% of the skin and no topical medicaments except for oily cream, B.P. or white soft paraffin, B.P. had been applied to the lesions for at least 1 week before biopsy specimens were taken. Attempts were made to assess the degree of activity of the psoriasis in each patient on the basis of clinical criteria, including the morphology, speed of development and number of skin lesions. The erythema threshold to ultraviolet radiation was determined in many of the patients and this was also used as a means of determining the activity of the disease as previously reported (Hodgson & Hell, 1972). Seven patients had acute, eruptive disease and seventeen had non-eruptive disease. The remaining ten patients were those who immediately prior to admission to hospital were in an eruptive stage, but at the time of biopsy a few days later were not developing any new lesions or extension of old ones; the term 'post-eruptive' was used to describe this group.

Epidermal biopsy specimens (Arthur & Shelley, 1959) were obtained under local anaesthesia (lignocaine) from psoriatic plaques (12 patients) and from macroscopically uninvolved skin (22 patients). All the specimens were taken from the posterior lower trunk and in the case of the clinically involved skin, the specimens were removed from the same psoriatic plaque. Three biopsy specimens were taken from each patient; the first specimen was ground in a cooled glass grinder, the latter being washed out with 1ml of Hanks' balanced salt solution and calf serum 7:3 containing tritiated thymidine 5μCi/ml, (36μCi/μm). This was immediately spun in a refrigerated centrifuge at 0°C at 3,000 rev/min for 6 min and the supernatant used as medium for one of the remaining skin specimens. The third specimen was floated on one ml of the same medium as was used for washing out the grinder. Both skin specimens were then incubated at 37°C for 4 h after which they were fixed and processed for autoradiography as described previously (Hell & Hodgson, 1966). Two thousand epidermal cells were counted on each specimen and the percentage of these showing more than twice the background number of grains calculated (usually 2–3 grains per cell minimum positive). All nucleated epidermal cells which were neither part of an appendage nor pyknotic were counted. Both specimens from each patient were processed together so that the results are strictly comparable and the methods used were standardized as detailed in previous communications from our laboratory (Hell & Cruickshank, 1963).

RESULTS

The percentage change in the proportion of epidermal cells in DNA synthesis after the addition of autologous extract is shown in Table 1. It should be pointed out that variations between identical pieces of skin taken at the same time are within 10%, hence the differences reported here are considerable. In both involved and uninvolved psoriatic skin, a stimulant effect of the added extract was observed when the disease was in an eruptive phase. The degree of stimulation was greater in the involved skin (109 ± 129) than in the uninvolved skin (33 ± 23). In non-eruptive psoriasis an inhibitory effect was apparent; this was more marked in the involved skin than in the uninvolved skin (40·5 ± 31. cf. 19 ± 24).

DISCUSSION

Inhibition of DNA synthesis in normal human epidermis by the addition of skin homogenates has been reported by Marks *et al.* (1971). They also showed that psoriatic skin extracts had a similar, but more marked, effect on both normal and psoriatic epidermis. Our results show that, although depression of DNA synthesis in psoriatic epidermis often occurs following the addition of autologous skin extracts, stimulation rather than depression of DNA synthesis is observed in some patients. Closer analysis of the results reported in this paper shows that there is an approximate correlation between the stage of activity of the psoriatic process and the effect of skin extracts on epidermal DNA synthesis. During the eruptive stage of the disease, skin extracts prepared from either the involved or clinically uninvolved skin stimulate DNA synthesis; when the psoriasis is in a more stable, non-eruptive stage, inhibition of DNA synthesis by the skin extracts is generally observed. The factors which determine the state of reactivity of the disease are largely unknown and accurate assessment of the degree of activity is difficult. In this study, eruptive and non-eruptive stages of psoriasis have been identified on the basis of clinical observations and the response to ultraviolet radiation tests. All the patients were hospitalized and many whose disease was in an eruptive stage at the time of admission,

TABLE 1. Effect of ground skin extract on autologous psoriatic epidermis *in vitro*. Proportion of cells in DNA synthesis after 4 hr *in vitro* without and with extract

Uninvolved					Involved				
Stage	Age and sex	Control	+Extract	% Change	Stage	Age and sex	Control	+ Extract	% Change
Non-eruptive	23F	6·6	4·2	↓ 36	Non-eruptive	22M	12·1	7·5	↓ 38
,,	47F	2·5	2·6	↑ 4	,,	54M	2·7	0·9	↓ 67
,,	63M	7·3	6·9	↓ 5	,,	62M	4·5	1·0	↓ 78
,,	53M	1·7	1·3	↓ 24	,,	53M	1·8	2·1	↑ 17
,,	69F	0·6	0·8	↑ 33	,,	30F	6·4	3·6	↓ 44
,,	51M	5·3	4·6	↓ 13		22M	0·9	0·6	↓ 33
,,	43M	2·1	0·8	↓ 62					
,,	45M	1·8	1·4	↓ 22					−40·5 ± 30
,,	28M	5·4	4·1	↓ 24	,,				
,,	24M	1·7	0·9	↓ 47					
,,	38M	2·2	1·8	↓ 18					
				−19 ± 24					
Post-operative	48F	1·0	0·8	↓ 20	Post-eruptive	48F	0·6	0·4	↓ 33
,,	62F	2·1	1·8	↓ 14	,,	34M	2·1	2·1	0
,,	50M	1·0	1·0	0	,,	25F	1·9	2·1	↑ 10
,,	52M	3·0	3·1	↑ 3					
,,	48M	2·4	2·2	↓ 8					−7·7 ± 18
,,	62F	2·4	1·0	↓ 58					
,,	29F	1·7	2·1	↑ 24					
				−10 ± 23					
Eruptive	34F	1·4	1·4	0	Eruptive	53F	13·4	21·8	↑ 63
,,	34M	1·1	1·6	↑ 45	,,	60M	5·1	7·1	↑ 39
,,	48M	1·1	1·8	↑ 64	,,	50M	0·4	1·3	↑225
,,	26M	1·7	2·1	↑ 24					
									+109 ± 129
				+33 ± 23					

Comparison of eruptive with non-eruptive and post-eruptive, $P < \cdot 01 > \cdot 001$. Students 't' test.

showed a rapid lessening of the activity of the psoriasis with bed rest and the application of bland emollients. Skin biopsy specimens were seldom taken within the first 48 h of admission to hospital; relatively few specimens were therefore obtained from patients whose psoriasis remained in an eruptive phase. After 48 h, the disease had become less active, the term 'post-eruptive' being applicable at this time. It is of interest that epidermal DNA synthesis during this post-eruptive stage was inhibited by autologous skin extracts, although not so marked as in the case of non-eruptive psoriasis.

There is increasing evidence that the state of activity of the psoriasis is an important consideration when interpreting clinical and experimental observations. Clinically, the isomorphic reaction is an indication that psoriatic skin behaves differently to trauma during the eruptive stage of the disease; attempts to elicit the reaction in non-eruptive psoriasis are usually unsuccessful. The decreased threshold to ultraviolet radiation when psoriasis is in an eruptive stage also reflects this difference in reactivity of psoriatic skin (Hodgson & Hell, 1972). Our results show that it is only during the phase of maximal activity of the disease that a high concentration stimulant effect on new cell production can be demonstrated by injuring epidermal cells; as soon as the psoriasis passes its peak of activity and during the non-eruptive stage, an inhibitory effect of this single high concentration of skin extract on DNA synthesis is observed. Previous studies have shown that the response to injury is decreased in subjects with non-eruptive psoriasis compared with non-psoriatic patients. This finding could be explained by the release of a factor inhibitory to DNA synthesis in approximately the same concentration as in normal skin, as the work of Marks *et al.* (1971) suggests, or could be explained as a concentration effect.

The concentration of ground skin used in the experiments reported in this paper is about a hundred times as high as in those reported in the paper on the stimulant action (Hell, 1970) and is more in line with Marks *et al.*'s (1971) concentrations. Even a potent stimulant fraction gives a lower proportionate effect if the concentration is increased above the optimum. This may be caused by toxic materials also present. In support of this concept, fractions from non-eruptive psoriatic skin diluted approximately a hundredfold will stimulate epidermal cells of non-psoriatic human skin to go into DNA synthesis (Hell & Hodgson, unpublished observations).

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Cytochemical identification of ATPase-positive Langerhans cells in EDTA-separated sheets of mouse epidermis

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SUMMARY

Sheets of epidermis for incubation to demonstrate ATPase activity were obtained from specimens of mouse footpad using EDTA as the separation medium. The use of EDTA in place of the NaBr method previously described, resulted in a greatly reduced incubation time, precise localization of reaction product and preservation of ultrastructural detail. A population of closely and regularly spaced ATPase-positive dendritic cells was demonstrated by light microscopy. Electron microscopy demonstrated that, with short incubation times, reaction product was found only in the extracellular space adjacent to dendritic cells, the majority of which possessed the typical ultrastructural features of Langerhans cells.

The histochemical method for demonstrating adenosine triphosphatase (ATPase) activity in mammalian epidermis using a modified Wachstein and Meisel medium (Wachstein & Meisel, 1957; Wolff & Winkelmann, 1967a) has frequently been used in light microscopic studies of the presence and distribution of Langerhans cells in normal, pathological and experimentally altered mammalian epidermis (Wolff & Winkelmann, 1967b; Lessard, Wolff & Winkelmann, 1968; Fritsch & Schellander, 1971; Fritsch, 1971; Molokhia & Portnoy, 1971; Brown, Winkelmann & Wolff, 1967; Reams & Tomkins, 1973). Wolff & Winkelmann (1967c) have presented cytochemical evidence for the specificity of this reaction for Langerhans cells in slices of skin and have demonstrated an absence of reaction product around keratinocytes and melanocytes in the presence of a strong reaction adjacent to the plasma membrane of Langerhans cells. Others have demonstrated a reaction for ATPase activity adjacent to the cell membranes of keratinocytes, melanocytes and neural elements (Zelickson & Mottaz, 1968; Bradshaw *et al.*, 1962; Riley, 1967; Mustakallio, 1962) and, consequently, the specificity of this method of identifying Langerhans cells has been questioned (Zelickson & Mottax, 1968). The demonstration of ATPase activity of cells clearly varies depending upon the technique employed, but Wolff (1972) has summarized the situation with the statement that the procedure of demonstrating the enzyme detailed in some publications (Wolff, 1967) is specific for the visualization of Langerhans cells within the epidermis.

Separated sheets of epidermis offer a useful way of investigating the spatial and numerical distribution of ATPase positive cells by light microscopy but, unfortunately, identification of cell types after the procedure of separation from the connective tissue with a solution of sodium bromide (Wolff & Winkelmann, 1967a,b; Lessard, *et al.*, 1968; Brown, *et al.*, 1967; Mackenzie, 1972) is confused by possible alterations in the histochemical reactivity of cells which may be produced by the separation media. The use of sodium bromide for separating the epidermis produces marked epidermal damage which prevents ultrastructural identification of cell types following incubation and also extends the incubation time necessary to demonstrate ATPase activity to more than an hour (Wolff & Winkelmann, 1967a,b).

Ethylenediamine tetraacetic acid (EDTA) can be used to separate sheets of epidermis from the connective tissue and the ultrastructural appearance of the separated sheets is well preserved (Scaletta & MacCallum, 1972). Sheets of mouse footpad epidermis were therefore prepared by separation with EDTA and incubated to demonstrate ATPase activity. After examination with the light microscope, some of the same sheets were processed for electron microscopy to identify the ATPase-positive cells and verify the specificity of the method.

MATERIALS AND METHODS

Specimens of skin from the glabrous part of the hind limb, just proximal to the footpad of adult male Balb/C mice, were incubated for 2 h at 37°C in buffered EDTA (Scaletta & MacCallum, 1972). After this treatment, the epidermis could be readily separated from the dermis with fine forceps and the epidermal sheets so obtained were fixed and incubated to demonstrate ATPase activity by a method similar to that of Wolff & Winkelmann (1967a,b). After separation, the epidermal sheets were: (a) washed in 3 changes of cold 0·2 M trismal buffer (with 6·84% sucrose) at a pH 7·3 for a total of 20 min; (b) fixed in cold (4°C) cacodylate buffered formaldehyde for 20 min; (c) re-washed in 3 changes of cold trismal buffer for a total of 30 min; (d) incubated at 37°C for 5, 10 or 15 min in a substrate containing 10 mg ATP (Sigma), 5 ml 5% $MgSO_4$, 3 ml 2% $PbNO_3$ in 42 ml trismal buffer (with 8·55% sucrose) at pH 7·3; (e) washed in buffer and treated with dilute ammonium sulphide.

Specimens for light microscopy were mounted flat in glycerin jelly. Specimens of both whole fresh footpad and of incubated epidermal sheets were prepared for electron microscopy by fixing for 1 h in glutaraldehyde/osmium mixture in 0·1 m cacodylate buffer (Hirsch & Fedorko, 1968) at pH 7·4 and 4°C, washed in saline for 30 min, post-treated with 0·25% uranyl acetate in 0·1 M acetate buffer at pH 6·3 for 20 min and rinsed in several changes of saline for 30 min. Specimens were dehydrated in ascending concentrations of acetone, passed through acetone/Epon mixtures and embedded in Epon.

For comparison of the results of the EDTA and NaBr methods of separation, epidermal sheets were obtained by treatment with 2N NaBr for 4 h at 20°C (Wolff & Winkelmann, 1967). Except that it was necessary to extend the incubation time to 30, 60 and 90 min to demonstrate ATPase activity, these specimens were subsequently treated in the same way as EDTA separated specimens.

Some specimens of intact skin were also fixed in formalin and processed by the usual histological methods for wax embedding and were sectioned and stained with haematoxylin and eosin.

RESULTS

Examination of sections of specimens processed for routine histological examination showed that the epidermis of the region examined possessed a thick stratum corneum, a well-formed stratum granulosum and six to seven layers of nucleated cells.

The electron microscopic examination of specimens of whole skin showed the normal features of

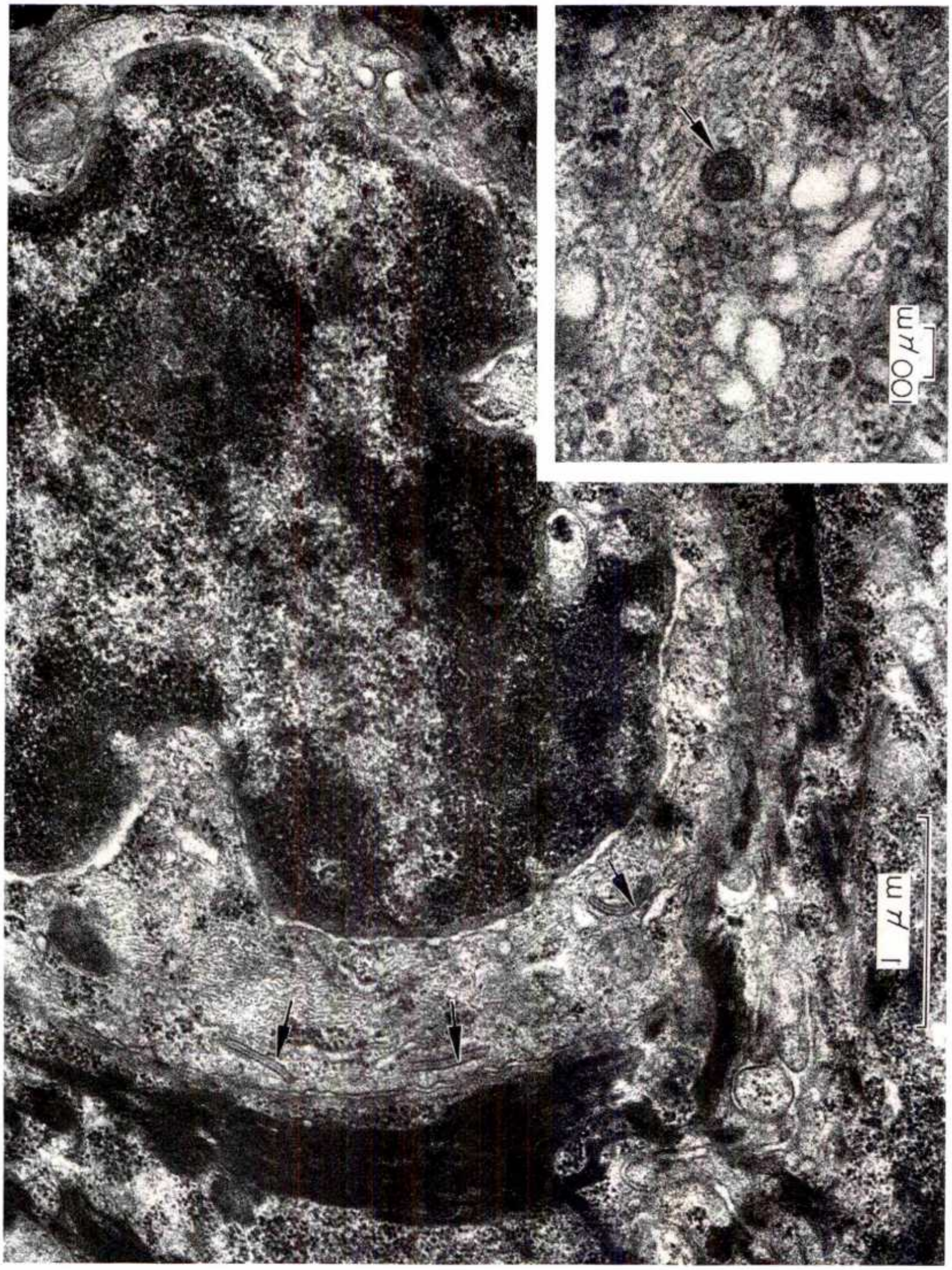

FIGURE 1. Electron micrograph of a Langerhans cell from the prickle cell layer of footpad processed by routine methods. Tonofibrils and desmosomes are lacking and the nucleus is indented. Several typical rod-shaped Langerhans granules are present (arrows). Inset: Portion of another Langerhans cell showing circular Langerhans granule (arrow) adjacent to a Golgi zone.

a keratinized epithelium. Interspersed between the keratinocytes of the lower part of the spinous layer, a regularly spaced population of cells was found which lacked tonofilaments and desmosomes and which possessed other features typical of the Langerhans cell such as an indented nucleus, a conspicuous Golgi region and lysosome-like vesicles (Fig. 1). The trilaminar granules characteristic of the Langerhans cell were also observed. As previously described (Birbeck, Breathnach & Everall, 1961; Sagebiel, Clark & Hutchins, 1971), these commonly appeared as straight rod-shaped profiles, but were occasionally curved or completely circular and sometimes expanded into a vesicle-like structure or were continuous with the plasma membrane.

EDTA separated sheets. Examination by light microscopy of the EDTA-separated, ATPase-incubated sheets showed deposition of reaction product in relation to a population of irregularly shaped cells which, by focusing through the epidermal sheet, were found to lie just suprabasally. These cells were regularly spaced, with a density of up to 2000/mm^2, and were usually dendritic. The number and morphology of the dendrites associated with each cell varied (Fig. 2). The majority of dendrites were short and blunt, but some extended for a distance equivalent to five to six basal cells from the main cell body. With short incubation periods (5 min), the reaction product was almost entirely

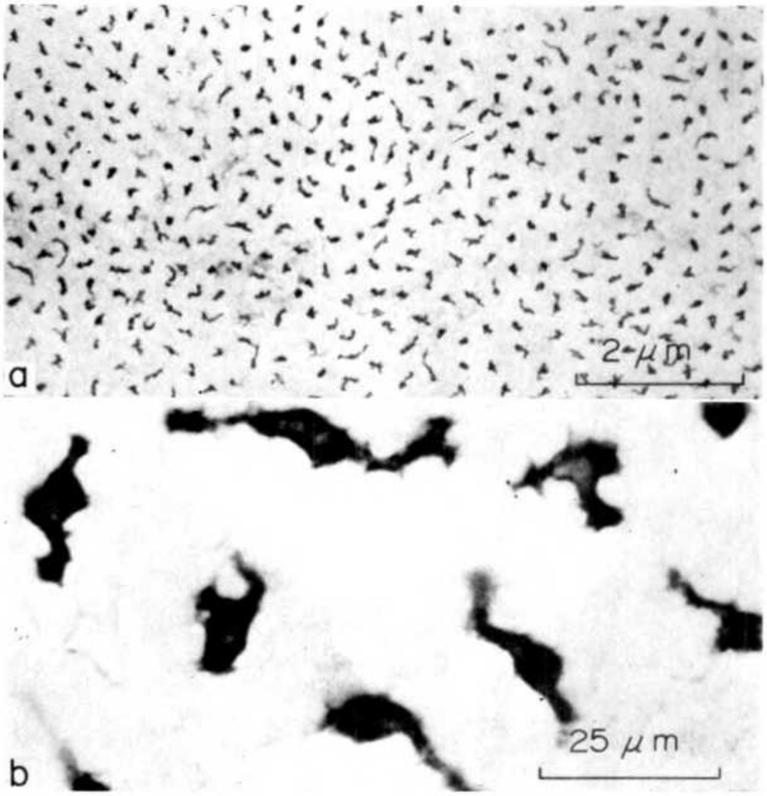

FIGURE 2. (a) Low power photomicrograph of sheet of footpad epidermis separated with EDTA and incubated (5 min) for ATPase activity showing numerous regularly spaced ATPase-positive cells.

(b) At higher power, it is seen that these cells possess dendrites of variable morphology and that a slight reaction product is just visible around the intervening keratinocytes. Bar represents 25 μm.

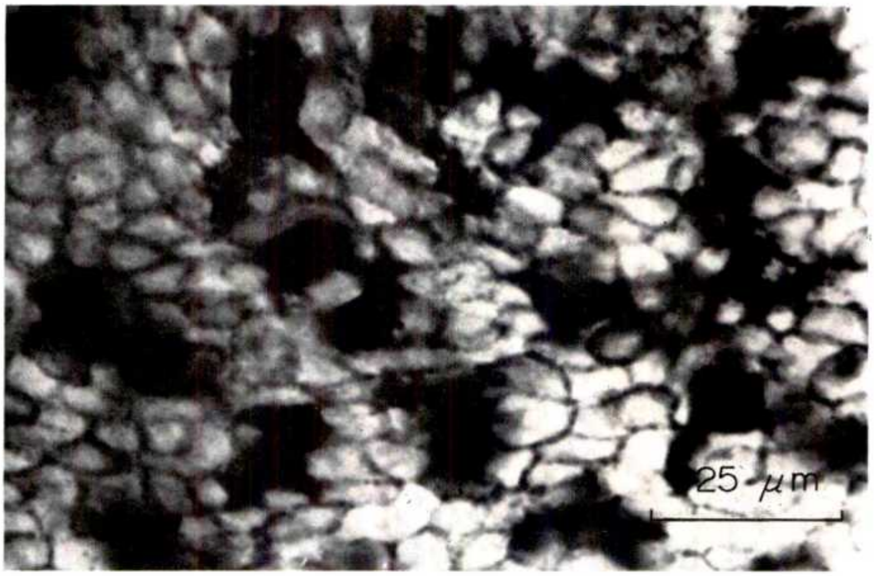

FIGURE 3. Photomicrograph of mouse epidermis separated with EDTA and incubated for 15 min for ATPase activity. Comparison with Fig. 2 shows a greater deposition of reaction product around dendritic cells but also deposition around intervening keratinocytes.

limited to the region of the membrane of such cells, but with increasing incubation time, outlines of keratinocytes also became reaction positive (Fig. 3).

Electron microscopy of the EDTA-separated, ATPase-incubated sheets showed that the histochemical processing had resulted in little loss of structural detail, and most of the ultrastructural features of the keratinocytes, such as tonofilaments, desmosomes and mitochondria, were clearly visible. A number of cells in the lower spinous layer showed a dense reaction product in relation to the outer surface of the plasma membrane (Fig. 4). These cells corresponded in number and spacing to the Langerhans cells identified in the specimens of unincubated epidermis. The cytoplasmic detail of these cells was not as well preserved as in keratinocytes and empty vacuoles and, on occasion, myelin figures were present in the cytoplasm. Structures clearly recognizable as Langerhans cell granules were, however, observed (Fig. 4) in sections of many of the cells. These showed a similar morphology to those described in normal tissue, although some granules appeared to have deteriorated as a result of the treatment to which the tissue had been subjected. Cells with the above morphology were never seen unaccompanied by reaction product.

NaBr separated sheets. Light microscopic examination of NaBr separated sheets after incubation for ATPase activity showed a pattern of distribution of reaction product recognizably similar to the EDTA separated sheets. However, a separate population of ATPase-positive cells was less well defined because all cell membranes reacted and a fine granular reaction product was scattered throughout the tissue. There was also some loss of cellular morphology as judged from the ATPase-positive cell outlines.

Sheets incubated for ATPase activity after NaBr separation showed considerable ultrastructural damage. Tonofilaments could be identified but plasma membranes, nuclear membranes and membrane bound structures such as mitochondria could not be defined. It appeared that the greater part of the

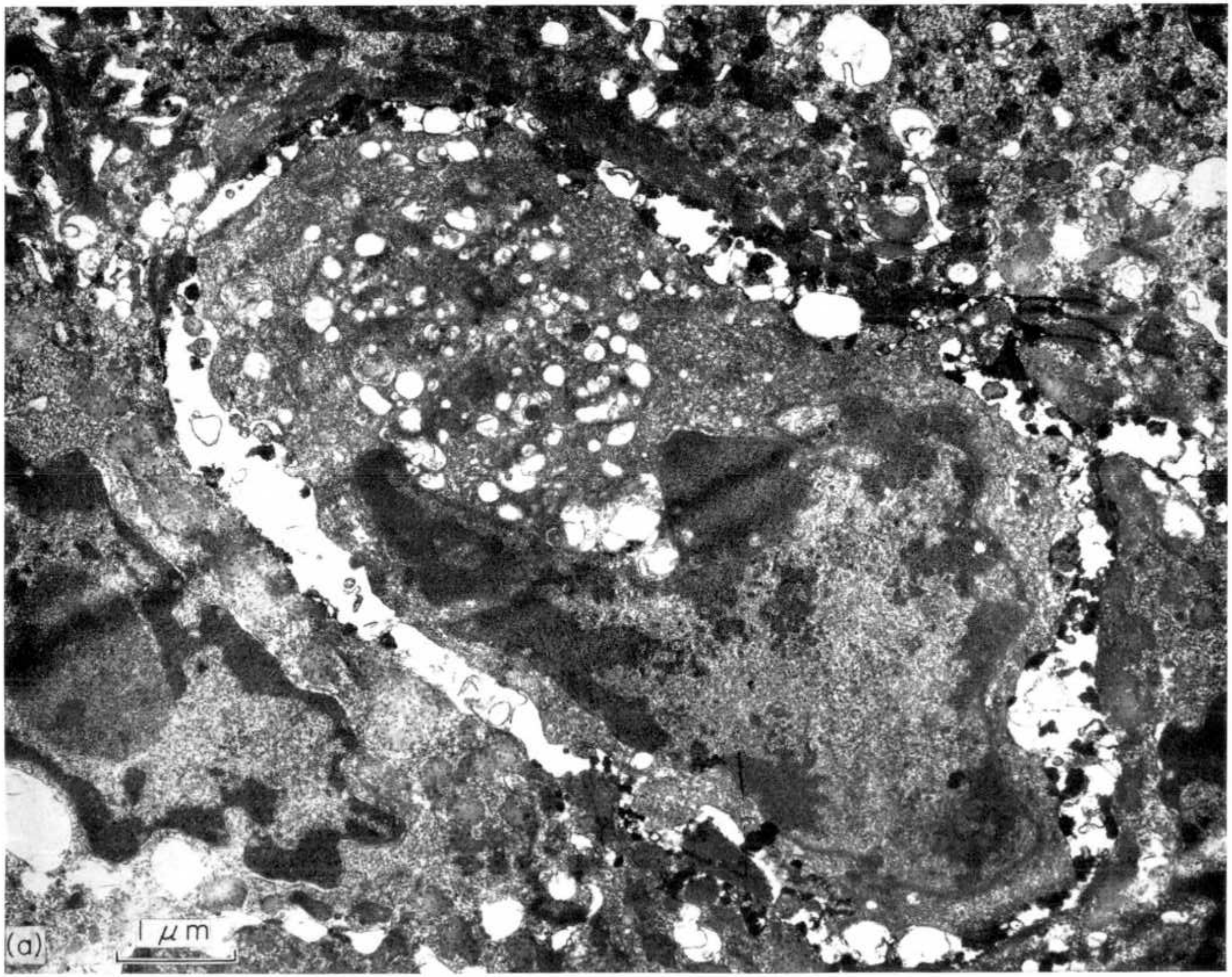

FIGURE 4. (a) Electron micrograph of EDTA separated footpad epithelium incubated to show ATPase activity. Reaction product is virtually confined to the intercellular space surrounding the Langerhans cells.

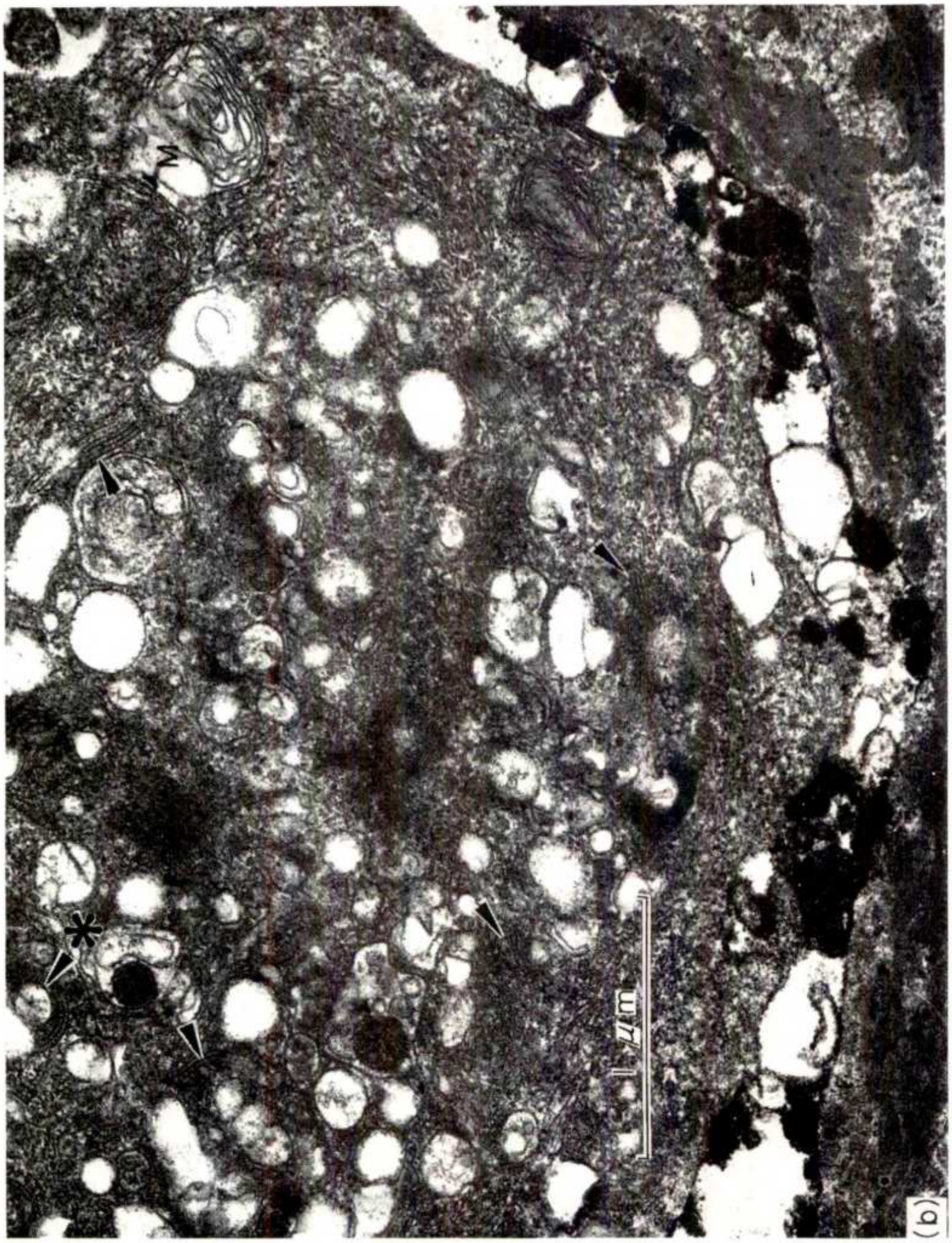

FIGURE 4. (b) Higher power micrograph of a portion of the Langerhans cell shown above. Parts of several Langerhans granules are evident (arrows) one of which is circular (asterisk). A myelin figure (M) is also apparent.

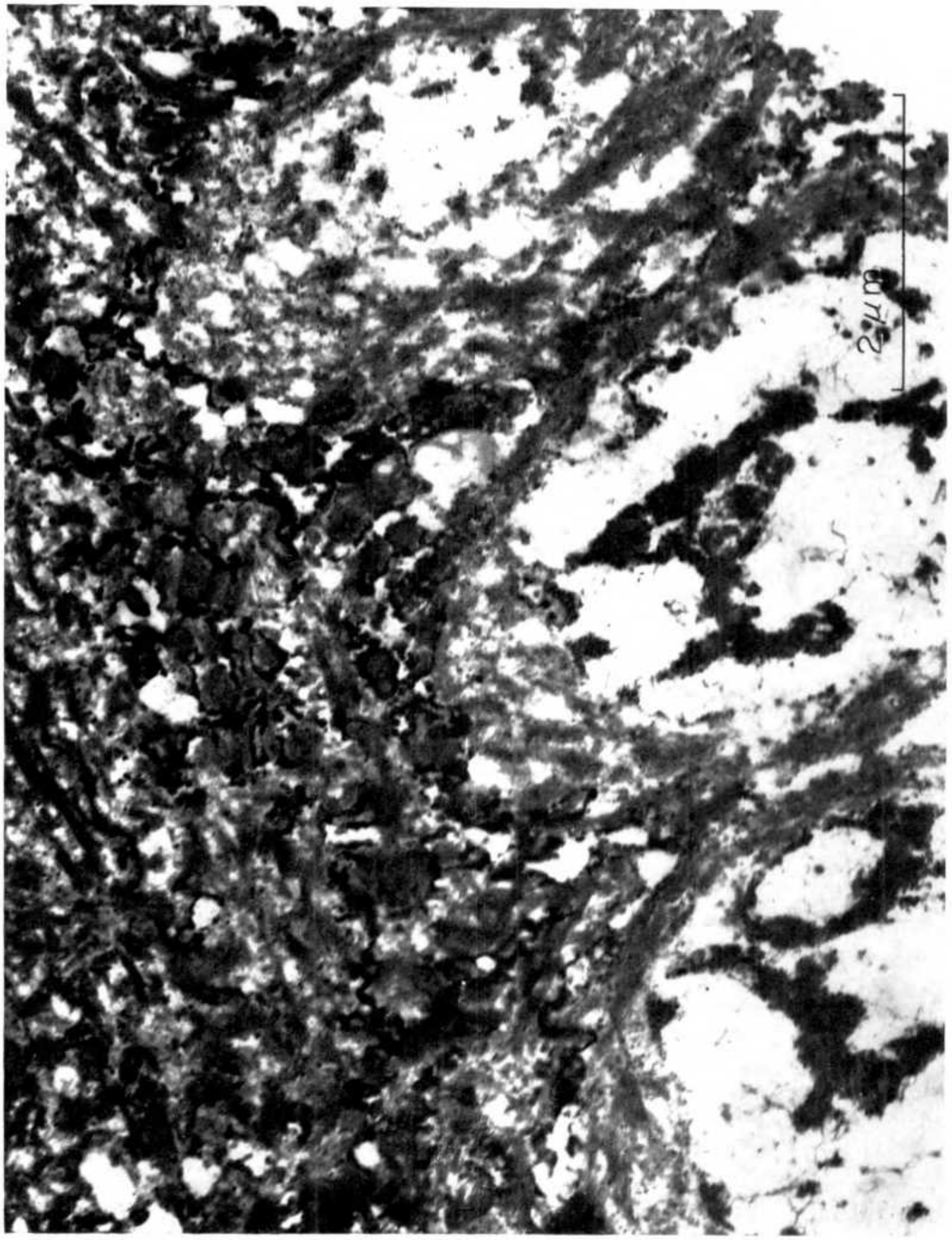

FIGURE 5. Electron micrograph of NaBr separated epithelium incubated to show ATPase activity. Reaction product is found in the region of the plasma membrane of the majority of cells and is scattered intracellularly. Membrane structures cannot be defined and other cytological features are poorly preserved.

intranuclear material was lost (Fig. 5). Reaction product was found in the region of the outer surface of the plasma membrane of the majority of cells and was also scattered intracellularly. Some cells could, with difficulty, be distinguished by a somewhat heavier peripheral reaction product and a very wide extracellular space. Cytological preservation, however, was inadequate for the identification but their distribution and position corresponded to the Langerhans cells observed in EDTA separated sheets.

DISCUSSION

The use of EDTA to obtain separated sheets of epidermis for studying the distribution of ATPase-positive cells appears to offer a number of advantages over the previous method of using sodium bromide. A comparison of the ATPase reaction in sheets of epidermis prepared for examination of light microscopy by separation with EDTA or by NaBr indicates that the use of EDTA leads to a more closely localized reaction product and better visualization of the cytology of dendritic cells. There is a great reduction in the incubation time, from the 1·5 h necessary after sodium bromide (Wolff & Winkelmann, 1967a), to 5–15 min after EDTA. Furthermore, use of EDTA sufficiently preserves cytological detail to allow ultrastructural examination of pieces of the same material as that used for light microscopic investigations.

The ultrastructural examination of sheets of mouse footpad epidermis incubated for ATPase activity provides grounds for identifying the ATPase-positive dendritic cells as Langerhans cells. The cells showing heavy deposits of reaction product adjacent to the plasma membrane corresponded in number, distribution and level within the epidermis to the Langerhans cells identified in specimens of unseparated epidermis. Though there was some loss of ultrastructural detail following separation and incubation, the degree of preservation was sufficient to identify a number of features typical of the ultrastructure of the Langerhans cells and, in particular, to demonstrate the presence of the characteristic intracytoplasmic Langerhans cell granule which is, at present, the definitive means of identifying these cells (Birbeck *et al.*, 1961; Sagebiel *et al.*, 1971). This granule was not invariably present in all cells showing a reaction, but this is to be expected for not every plane of a section can include profiles of such a small organelle; in all other respects, the ultrastructure of the reactive cells was similar. No evidence of non-reactive dendritic cells was found.

The demonstration of Langerhans cells in the epidermis of the hairless region proximal to the mouse footpad appears to be of interest to two suggestions which have been made concerning the function of this cell. It has been suggested that Langerhans cells may contribute to hydrolytic enzymes necessary to achieve the more complete keratinization found at other body sites (Jarrett & Spearman, 1964). However, the histological appearance of the thickened epidermis of the region of the mouse footpad examined is typical of that of plantar and palmar surfaces which, it has been suggested, show less hydrolytic activity (Jarret & Spearman, 1964), but the number of Langerhans cells per unit area found in the epidermis of this region is almost twice that found in the thinner epidermis of the mouse ear (Mackenzie, 1972). The presence of the large number of Langerhans cells in the epidermis of the footpad also goes against suggestions of an inverse relationship between the number of Langerhans cells and epidermal mitotic rate (Sagebiel *et al.*, 1971), as the labelling index and rate of regeneration of the epidermis of the mouse footpad have been shown to be higher than those of the thinner epidermis at other sites (Christophers & Lawrence, 1973; Mackenzie, 1974).

The presence of reaction product around keratinocytes with the increasing incubation times is of interest in relation to previous objections to the specificity of this technique, and demonstrates that there is no apparent qualitative difference in the reaction of the plasma membranes of keratinocytes and Langerhans cells after incubation for ATPase activity (Bradshaw *et al.*, 1962). However, with both long and short incubation times, Langerhans cells showed a markedly greater degree of reaction which resulted in quantitative differences that were quite adequate for these cells to be clearly distinguished from keratinocytes by light microscopy. Melanocytes were not observed in any of the specimens of mouse footpad epidermis examined and, therefore, results of the present work cannot relate to this aspect of the specificity of the reaction. The confused situation with regard to the relationship of Langerhans cells to non-specific or α-dendritic cells (Reams & Tomkins, 1973; Sagebiel, et al., 1971; Hutchins, Sagebiel & Clark, 1971; Mishima, Kawasaki & Pinkus, 1972) was not specifically examined. In a recent review, Wolff (1972) has suggested that with the appropriate procedure, the ATPase method is specific for the visualization of Langerhans cells. The present study supports that view and suggests that this specificity of visualization is maintained after separation with either NaBr or EDTA. However, the use of EDTA as the separating agent possesses a number of marked advantages over the use of NaBr.

ACKNOWLEDGMENTS

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The effect of transfer factor on the *in vitro* migration of tuberculin stimulated white cells from sarcoid and normal subjects

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SUMMARY

In the mixed leukocyte migration test, white cells from Mantoux positive and Mantoux negative individuals and from patients with sarcoidosis responded similarly to tuberculin, there being no significant difference in the migration index of the three groups. The cells from patients with sarcoidosis, however, showed a depressed response to phytohaemagglutinin. Transfer factor prepared from an individual with a very positive Mantoux reaction did not have any significant effect on the migration index when added to the white cells from sarcoid patients either in the presence or absence of tuberculin.

In 1945 Chase found that delayed hypersensitivity could be transferred from tuberculin sensitive to tuberculin negative guinea-pigs by means of cell exudates. Lawrence (1955) subsequently confirmed this finding in man using a leukocyte extract.

Many investigations have been made on the nature and immunological properties of the substance known as transfer factor present in leukocyte extracts (Baram & Mosko, 1962; Levin *et al.*, 1970; Kirkpatrick, Rich & Smith, 1972).

Transfer factor is a low molecular weight substance obtained by dialysis of white cells which have been ruptured by freezing and thawing. When prepared from the cells of individuals showing strongly positive skin reactions to candida antigen and given to patients with chronic muco-cutaneous candidiasis, it has the capacity to restore skin sensitivity to candida and temporarily improves the condition of the patient (Valdamarsson *et al.*, 1972; Pabst & Swanson, 1972). In sarcoidosis, Lawrence & Zweiman (1968) have shown that local sensitivity to tuberculin can be transferred by means of transfer factor produced from strongly positive tuberculin reactors.

In 1932 Rich & Lewis found that adding tuberculin to explants of spleen and lymph nodes from tuberculin sensitive animals inhibited migration of these cells. This method has been modified by George & Vaughan (1962) using peritoneal exudate cells and later by Søborg & Bendixen (1967) who used capillary tubes containing peripheral leukocytes instead of exudate cells, i.e. the leukocyte migration inhibition test. Thor (1967) found that it was possible to correlate *in vitro* inhibition by tuberculin with a positive tuberculin skin test. The migration inhibition reaction was further correlated with the size of the intracutaneous reaction to tuberculin by Clausen & Søborg (1969). There have, however, been other less consistent reports on the correlation between *in vitro* migration and *in vivo* reactions to PPD (Kaltreider *et al.*, 1969; Rosenberg & David, 1970).

Sarcoidosis, being a systemic granulomatous disease in which depression of delayed sensitivity is shown to tuberculin as well as to candidin (Friou, 1952), histoplasmin (Israel *et al.*, 1952), brucellin, coccidioidin and mumps antigen (Hoyle, Dawson & Mather, 1954; Quinn, Bunch & Yagle, 1955) would seem, therefore, a suitable subject for the *in vitro* study of transfer factor using leukocyte migration. This is the subject of the present study.

MATERIALS AND METHODS

Seventeen patients with a clinical history of sarcoidosis were investigated. Tissue and/or Kveim test histology confirmed the diagnosis in fourteen of the patients. The remaining three were diagnosed on clinical history and radiological evidence alone. The mean age of the patients was 46 years, the range being from 30 to 70 years. Thirteen age and sex matched controls were also investigated. Thirteen of the sarcoid and three of the normal patients were Mantoux negative, the remainder being Mantoux positive. A positive reactor was taken to be one who produced an area of oedema 10 mm in diameter 48 h after the injection of 0.1 ml of old tuberculin (100 units per ml).

Leukocyte culture

Twenty-five millilitres of peripheral blood was collected and quickly transferred to plastic tubes each containing 0·3 ml preservative-free heparin. The leukocyte migration inhibition test (LMI) (Søborg & Bendixen, 1967; Søborg, 1971) was then carried out as described by Morison (1974). The migration

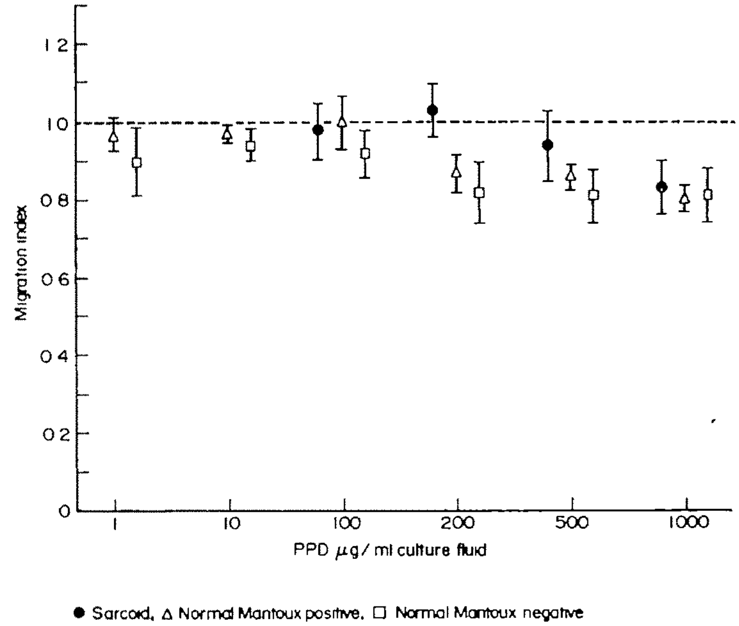

FIGURE 1. Leukocyte migration inhibition test of normal, Mantoux positive, Mantoux negative and sarcoid subjects challenged with varying concentrations of PPD per ml culture fluid. Limits given are mean ± s.e. mean.

index (MI) is defined as the ratio between the mean area of migration in the presence of antigen and the mean area of migration in the control chambers. Each test was performed in triplicate and the results were only included when the difference between chambers was less than ± 10%.

A migration index of 15–20% was taken as an indication of significant stimulation or inhibition of migration as compared with the control.

Antigen

Purified protein derivative of tuberculin (PPD dried powder, Weybridge) was used at concentrations varying between 1 and 1000 μg/ml culture fluid. A fresh stock dilution of 10 mg PPD in 0·85% NaCl was prepared each day. Phytohaemagglutinin (PHA Burroughs Wellcome, freeze dried grade) was used as a positive control for inhibition of migration at a concentration of 5 μg/ml culture fluid (Morison, 1974). A similar volume of 0·85% NaCl was used for the control migration.

Transfer factor

Three samples of transfer factor were obtained: one prepared from four adult blood donors (given by Dr Valdimarsson), one from 450 ml blood from a very strongly positive tuberculin reactor and one from a negative tuberculin reactor—the last two samples being prepared by the method of Lawrence & Askari (1971).

Each of the samples of transfer factor were made up so that the amount contained in 0·05 ml was equivalent to the transfer factor derived from 1×10^7 leukocytes.

Transfer factor obtained in this way has been shown to possess *in vitro* activity.

RESULTS

When the white cells from Mantoux positive and Mantoux negative individuals and from sarcoid patients were stimulated with PPD in the leukocyte migration inhibition test using 1, 10, 100, 200, 500 and 1000 μg PPD/ml culture fluid, no significant difference in the migration index was noted between the three groups of cells (Fig. 1).

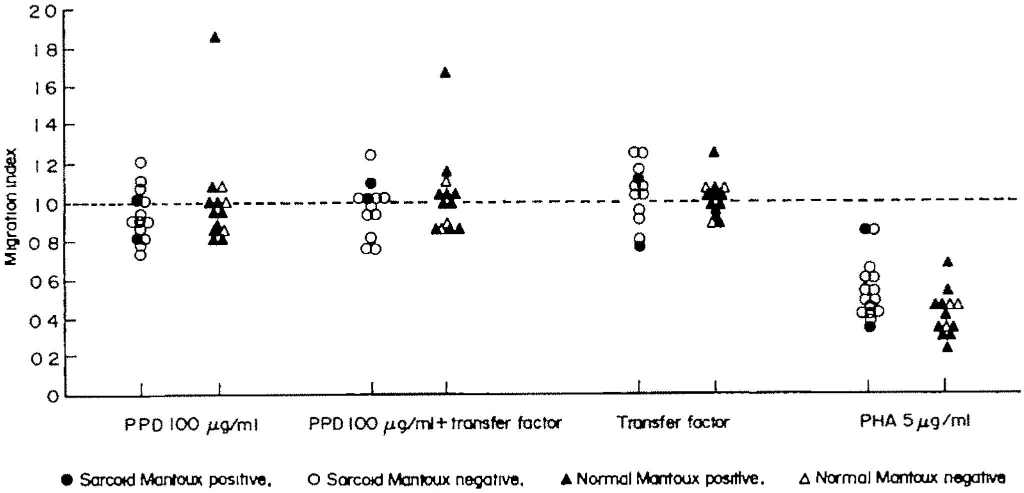

FIGURE 2. The effect of PPD, transfer factor from four adult donors and PHA on the migration of leukocytes derived from Mantoux positive and Mantoux negative patients with sarcoidosis and normal subjects.

The addition of transfer factor made from both Mantoux positive and Mantoux negative individuals had no effect on the migration of leukocytes from sarcoid and from normal Mantoux positive and negative individuals either alone or with PPD in the culture medium (Fig. 2, Table 1). As seen in this figure, white cells from patients with sarcoidosis were less inhibited when incubated with phytohaemagglutinin in the culture medium than the cells from normal Mantoux positive and Mantoux negative individuals.

TABLE 1. A comparison of the effect of transfer factor (TF) from Mantoux positive and Mantoux negative subjects with and without PPD on the white cells from Mantoux positive normal subjects and Mantoux negative patients with sarcoidosis in the leukocyte migration inhibition test

	Mean migration index ± s.e. mean	
Stimulus	Sarcoid	Normal control
Positive TF	1·09±0·13	1·12±0·23
Negative TF	1·10±0·25	1·21±0·27
PPD	0·94±0·14	1·01±0·27
PPD+positive TF	1·02±0·18	1·03±0·20
PPD+negative TF	1·0 ±0·30	1·04±0·20
TF from pooled adult blood	1 03±0·04	1·01±0·03
TF from pooled adult blood+PPD	0·97±0 04	1·02±0 06

DISCUSSION

Since it has been shown that transfer factor can partially reconstitute tuberculin sensitivity *in vivo* (Lawrence & Zweinman, 1968), we thought that it would be interesting to see what effect transfer factor, prepared from an individual with a positive tuberculin skin reaction, would have *in vitro* on the leukocytes in the mixed leukocyte migration inhibition test. Correlation of skin tests with *in vivo* response to PPD using the LMI test has been inconsistent. Rosenberg & David (1970), although initially unsuccessful, after some modification of the test obtained correlation in 92% of subjects when using 100 μg PPD/ml culture fluid. This agreed with an earlier observation of Thor *et al.* (1968). Becker *et al.* (1972) confirmed the known impairment in sarcoidosis of cellular immunity to antigens such as PPD using the *in vitro* LMI test. The impairment, however, seemed to be antigen dependent as migration inhibition factor and transfer factor are still produced by the lymphocytes.

The results of our experiments show no significant inhibition of migration by PPD in the Mantoux negative sarcoid subjects. In normal controls, however, there was no correlation between leukocyte migration and skin tests. This confirms the work of Becker *et al.* (1972) who found no agreement between *in vivo* and *in vitro* tests with PPD in normal controls. Kaltreider (1969) was similarly unable to correlate *in vitro* and *in vivo* results using 0·1–250 μg PPD/ml culture medium. He concluded that the observed apparent migration depended on the force of gravity abetted by minimal random cellular motion.

Transfer factor has been successfully used *in vivo* for the treatment of chronic muco-cutaneous candidiasis (Buckley *et al.*, 1968; Rocklin *et al.*, 1970; Valdimarsson *et al.*, 1972). Fireman *et al.*

(1967) showed that the addition of PPD to leukocytes obtained from Mantoux negative individuals and incubated with transfer factor from positive skin reactors gave similar results to PPD stimulated leukocytes from Mantoux positive individuals. We have found that the addition of transfer factor, made from PPD positive donors, to PPD stimulated lymphocytes derived from patients with sarcoidosis does not alter the migration of cells when using the LMI test. Also the addition of transfer factor to normal PPD stimulated lymphocytes from Mantoux negative and positive individuals did not enhance the effect of PPD alone.

We found also that the inhibitory response induced by PHA in leukocytes from sarcoid patients was slightly less than the response of leukocytes from normal persons. Other authors have also shown that leukocytes of sarcoid patients are less susceptible to PHA than those from normal persons (Hirschorn *et al.*, 1964; Topilsky 1972; Morison, 1973).

Our experiments demonstrate that transfer factor prepared from white cells from a Mantoux positive individual did not transfer *in vitro* sensitivity to white cells from Mantoux negative individuals nor to the white cells from patients with sarcoidosis when attempts were made to stimulate these cells with tuberculin as judged by the inhibition of leukocyte migration.

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The sebum excretion rate in rosacea

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SUMMARY

The sebum excretion rate (SER) was measured in fifty-five patients with rosacea and 126 control subjects. The mean SER in the patients with rosacea was not increased, nor was there any correlation between SER and severity of rosacea. Our data suggest that seborrhoea plays no part in the pathogenesis of rosacea.

The wealth of unsubstantiated hypotheses regarding the aetiology of rosacea varies from 'something alkaline in the stomach to something horrid in the cow-shed' (Borrie, 1953). An association between seborrhoea and rosacea has been claimed (Stokes & Beerman, 1932; Beerman & Stokes, 1934) and refuted (Klaber & Wittkower, 1939; Søbye, 1950; Marks, 1968) and many modern textbooks still suggest that seborrhoea is implicated in the pathogenesis of rosacea (Jarrett, Spearman & Riley, 1966; Church & Sneddon, 1971; Domonkos, 1971; Hall-Smith, Cairns & Beare, 1973). We have found no reports of quantitative studies of the sebum excretion rate in rosacea, and so we here report the result of such a study.

METHODS

The sebum excretion rate (SER) from forehead skin was measured by a gravimetric method (Cunliffe & Shuster, 1969a) in twenty-six male and twenty-nine female patients with rosacea, who attended the Skin Out-patients Department. The severity of the rosacea was graded as mild, moderate or severe, and the treatment was recorded. For comparison the SER was also measured in 126 patients of various ages attending the Skin Out-patients Department for treatment of localized dermatoses, such as warts and nail dystrophy. Patients with a previous history of acne vulgaris were not excluded from either group of patients, since this would have biased our results if rosacea has a positive or negative association with acne vulgaris.

RESULTS

Since the SER varies with age and sex (Cunliffe & Shuster, 1969b) the patients were divided into three age-groups for each sex (20–39, 40–59 and 60–79 years). The results (Table 1) show clearly that rosacea is not associated with seborrhoea, since the mean SER in the rosacea patients did not differ significantly from the SER in any of the corresponding group of controls.

Table 2 shows the mean SER's expressed as a percentage of normal values for the corresponding age and sex in patients with rosacea of varying degrees of severity. The SER was not significantly increased in any of the three groups studied, and the value was lower in the severe cases than in the mild cases, though this difference was not significant. We conclude that there is no correlation between SER and the severity of rosacea.

TABLE 1. Sebum excretion rates ($\mu g/cm^2/min$) in rosacea and control patients

	AGE (years)											
	20–39				40–59				60–79			
	Mean	s.e.	*n*		Mean	s.e.	*n*		Mean	s.e.	*n*	
Males												
Rosacea	1·31	0·29	7	n.s.	1·29	0·13	11	n.s.	0·65	0·08	8	n.s.
Controls	1·31	0·12	15		1·00	0·08	15		1·07	0·13	19	
Females												
Rosacea	0·85	0·22	9	n.s.	0·65	0·10	16	n.s.	0·31	0·05	4	n.s
Controls	0·66	0·07	30		0·54	0·07	31		0·24	0·05	16	

TABLE 2. Sebum excretion rates expressed as percentage of normal for age and sex in patients with rosacea of various degrees of severity

Excretion rate	Mean	s.e.	*n*
Mild	122·0	24·2	15
Moderate	106·9	14·7	24
Severe	106·8	8·2	16

Twenty-one patients with rosacea were taking tetracycline at the time of the SER estimation. The mean SER (as a percentage of normal) in these patients was 112·4±s.e. 17·5% compared with 109·7 ±s.e. 10·9% in the thirty-four patients not receiving tetracycline therapy, and this difference was not significant.

DISCUSSION

We have shown that the SER on the forehead is not increased in patients with rosacea, and this suggests that seborrhoea is not important in the pathogenesis of rosacea.

The authors of clinical studies who have reported a high incidence of seborrhoea in patients with rosacea may have misinterpreted the shiny forehead skin of rosacea, which is probably due to a combination of erythema and lymphoedema. In some of our patients the forehead looked shiny even though it did not feel greasy, and the SER subsequently proved to be normal or low. The presence of rhinophyma in some patients, with dilated follicular orifices on the nose, also contributes to a clinical impression of seborrhoea. We measured the SER only from forehead skin, and the possibility remains that rhinophyma is associated with seborrhoea of the nose. It is equally possible that the sebaceous glands of the nose may be enlarged without a corresponding increase in sebum excretion. In any case our normal SER measurements were found in skin which was affected by the disease, and this suggests that acne rosacea, unlike acne vulgaris (Cunliffe & Shuster, 1969b; Burton & Shuster, 1971) is not generally associated with seborrhoea.

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Erythropoietic protoporphyria—submicroscopic events during the acute photosensitivity flare

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SUMMARY

The immediate response of erythropoietic protoporphyria (EPP) skin to long-wave ultraviolet radiation (UVR) was studied with the electron microscope. The main finding was severe vascular injury. This was confined to the superficial vessels of the dermis and consisted of endothelial cell degeneration and a pronounced leakage of vascular contents. In contrast, the epidermis showed no abnormalities. Short-wave UV irradiation of EPP skin resulted in epidermal changes typical for the usual sunburn reaction and spared the dermal blood vessels.

The following conclusions are drawn: (i) Endothelial cells are the primary cellular target for the photodynamic reaction in EPP. (ii) The fibrillar material, characteristic for chronic EPP lesions, originates from the vessels and vascular contents. (iii) The multilayered basement membranes observed in such lesions reflect multiple consecutive reparative processes that follow endothelial injuries.

The clinical hallmark of erythropoietic protoporphyria (EPP) is photosensitivity to long-wave UV radiation, the responsible wave-lengths being identical to the absorption spectrum of protoporphyrin (Magnus *et al.*, 1961). The manifestations of the acute flare are oedema, erythema, and occasionally purpura whereas chronic lesions present as thickening and furrowing of the skin as well as superficial scarring (Peterka, *et al.*, 1965). The histopathological and electron microscopical changes in chronic EPP lesions are well known (Ryan, 1966) but, to our knowledge, there is no information on the cellular and subcellular alterations that occur during an acute photosensitivity flare. Since such data might help to pinpoint the primary cellular target of the photodynamic reaction a study was designed to investigate the initial response of EPP skin to UVR. The present paper deals with the ultrastructural changes observed in EPP skin during the immediate response to solar irradiation and to the long-wave UV spectrum of an artificial light source.

MATERIALS AND METHODS

Two patients with EPP, a 21-year-old man and an unrelated woman, aged 34 years, were selected for the study. Both exhibited high red blood cell (RBC) protoporphyrin levels (1680±521 $\mu g\%$ and

2020±290 μg%, respectively, in the absence of porphyrinuria), RBC fluorescence, and photohaemolysis. Needle liver biopsies of both patients presented signs of moderate liver pathology and protoporphyrin crystals were found, by electron microscopy, in the hepatocytes and Kupffer cells (Wolff, Wolff-Schreiner & Gschnait, 1974). Both gave a history of severe photosensitivity since infancy. They presented skin lesions with the characteristic histological (Rimington, *et al.*, 1967; Ryan, 1966), histochemical (Ryan, 1966), and immuno-histochemical (Epstein, Tuffanelli & Epstein, 1973) changes.

Irradiation with sunlight

H.H. was exposed to sunlight for 2 h at noon on a summer day which was slightly cloudy. Two and a half hours after the exposure the patient noted a burning sensation in the exposed sites and 30 min thereafter developed oedema and erythema.

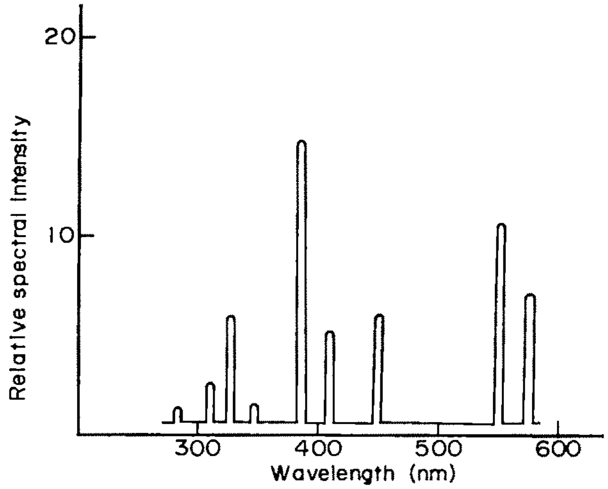

FIGURE 1. Relative spectral output in the UV range of the Osram Ultravitalux 300 lamp used in this study.

Irradiation with artificial light

A bank of four Osram Ultravitalux 300 lamps, whose spectral UV output is shown in Fig. 1, was employed as a light source. At an operational distance of 40 cm the mean minimal erythema dose (MED) was found to be 4 min in the patients and in matched healthy volunteers.

(i) *Irradiation with the unfiltered lamp* (*UVB irradiation*). Test sites, measuring 5 cm in diameter and located on the upper arms of the patients, were irradiated for 10 min at a light-target distance of 40 cm. This dose represented 2½ MED and caused considerable erythema in both patients.

(ii) *Irradiation with the glass-filtered lamp* (*UVA irradiation*). Fields measuring 5 cm in diameter on the forearms and dorsa of the hands of the patients were irradiated as above but a 3 mm glass filter was interposed to block wavelengths below 310 nm. Irradiation was continued until the patients noted mild burning or itching and was then stopped. Exposure times were 165 min for H.H. and 180 min for B.D. Within 2 h after exposure both patients developed mild oedema and erythema within the irradiated sites.

Biopsies

Shave biopsies of the irradiated skin sites were performed 2½ and 16 h after irradiation with the artificial light source and 2½ h after sun exposure. Shave biopsies were also taken from non-irradiated exposed regions of the body.

Laboratory procedures

Biopsy specimens were fixed and processed for routine histology (haematoxylin and eosin, and PAS staining of paraffin-embedded specimens) and electron microscopy. The latter included fixation in a 1:1 dilution of the paraformaldehyde-glutaraldehyde fixative of Karnovsky (1965) for 5 h at room temperature; rinsing in 0·1 m cacodylate buffer at 4°C; post-fixation in 3% aqueous osmic acid; and after rapid dehydration in a graded series of alcohols, embedding in Epon 812. Ultrathin sections were cut with diamond knives (Dupont, Dehmer), using LKB ultrotome III and Reichert OM U2 ultramicrotomes, and stained with lead citrate; they were examined with a Zeiss EM 9S electron microscope.

RESULTS

Unirradiated controls

Light exposed skin sites. Specimens taken from chronically sun-exposed skin sites exhibited all the characteristic features of a chronic EPP lesion (Ryan & Madill, 1968; Anton-Lamprecht & Bersch, 1971). At the light microscopic level the papillary and subpapillary vessels appeared hyalinized and ensheathed by a homogeneous eosinophilic mass that also filled an occasional dermal papilla. At the ultrastructural level there were multiple reduplications of the vascular basement membranes that surrounded the vessels in a concentric fashion (Fig. 2). The vessels, including their basement membranes, were embedded in a mass of finely fibrillar material that contained occasional foci of cellular debris but was otherwise strikingly acellular.

The endothelial cells of these vessels appeared intact exhibiting the usual number of well developed cell organelles, endothelial granules, microfibrils, and pinocytotic vesicles. Pericytes, fibroblasts, and epidermal cells were normal but there was some hyperkeratosis and the granular layer appeared thickened.

Irradiated skin

Sun light. The epidermis appeared completely normal and showed none of the cytological features characteristic for a 'normal' sunburn reaction of the epidermis (Nix *et al.*, 1965; Hönigsmann, Wolff & Konrad, 1974).

The dermis exhibited striking abnormalities that were superimposed on the chronic EPP changes described above and were confined to the subepidermal capillaries and the small pre- and post-capillary vessels. These vessels were packed with red blood cells (Fig. 3) and contained a substance with a foamy appearance that, at higher magnifications, was found to consist of an amorphous, granular material, fine fibrils, innumerable vesicles of various size, and residues of membranes (Fig. 3). The endothelial cells presented the most striking alterations—their cytoplasm was highly vacuolated assuming a Swiss-cheese-like morphology (Figs 3–5). Cytoplasmic organelles that were still recognizable showed tremendous swelling and what was left of the cytoplasm proper was condensed to dense intervacuolar strands that were composed of tightly packed cytoplasmic filaments (Fig. 4). The nuclei of these cells appeared condensed and frank cytolysis was often observed.

The vessels were dilated; the damaged endothelial cells had frequently lost intercellular contact and leakage of the intravascular contents had occurred through the gaps thus formed (Figs 3 and 5).

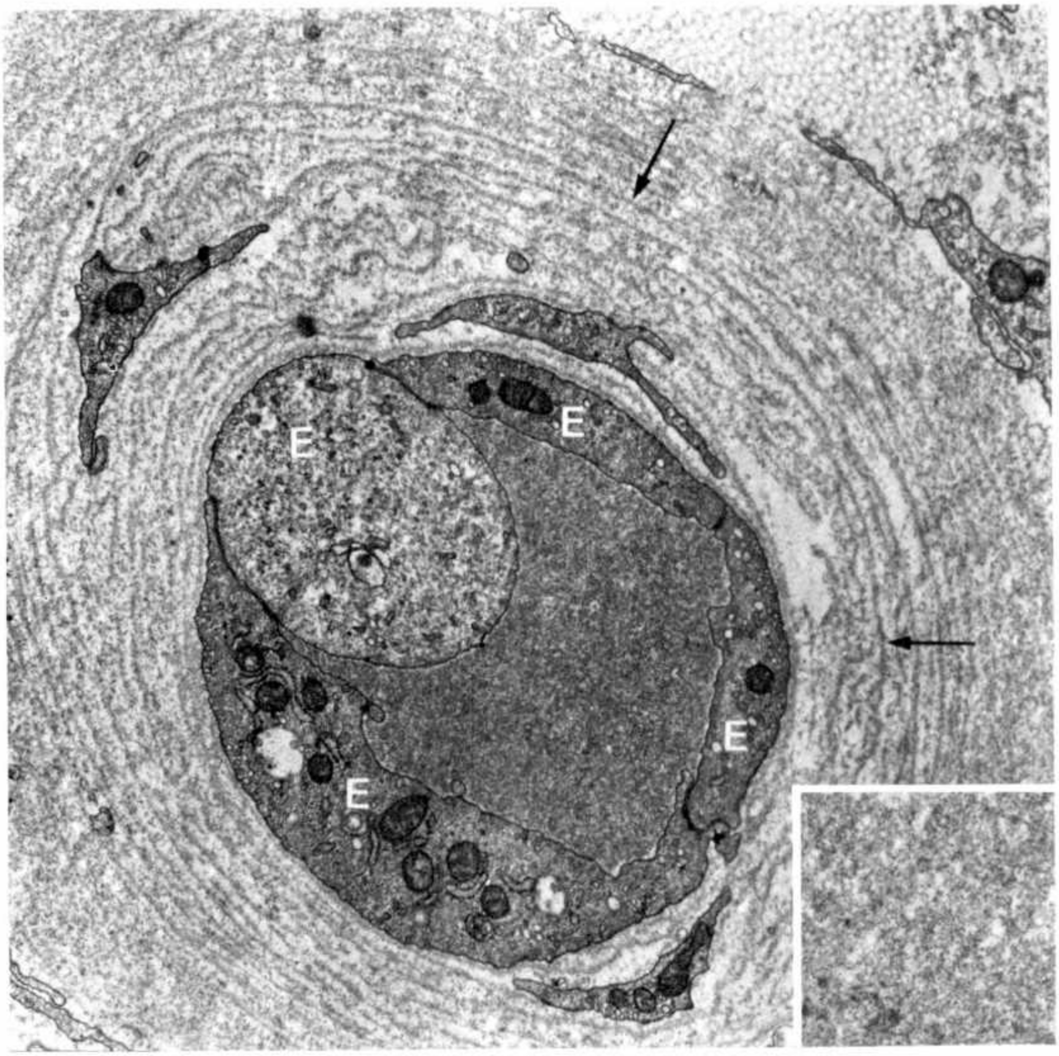

FIGURE 2. Biopsy from the dorsum of the hand of H.H. Note the multiple basement membranes (arrows) surrounding the papillary blood vessel. Inset shows the fibrillar material typical for chronic EPP skin lesion. E, endothelial cell (× 12,000; inset × 22,200).

Red blood cells were found penetrating these openings in the capillary walls, breaking through the basal lamina, and lying outside the vessels within the fibrillar perivascular masses and between the multiple basal laminae (Figs 3 and 5). Some of these cells showed features of haemolysis. The foamy material observed within the vascular lumen also extended into the perivascular tissue where it surrounded the vessels that were thus literally bathed in this substance (Figs 3 and 5). The pericytes and fibroblasts, however, appeared normal. Phagocytosis of the foamy material by macrophages was occasionally observed (Fig. 3) and numerous mast cells in the perivascular tissue were degranulated.

Artificial light

(a) *Reaction to UVB* ($2\frac{1}{2}$ MED delivered by the unfiltered lamp). Two and a half hours after irradiation

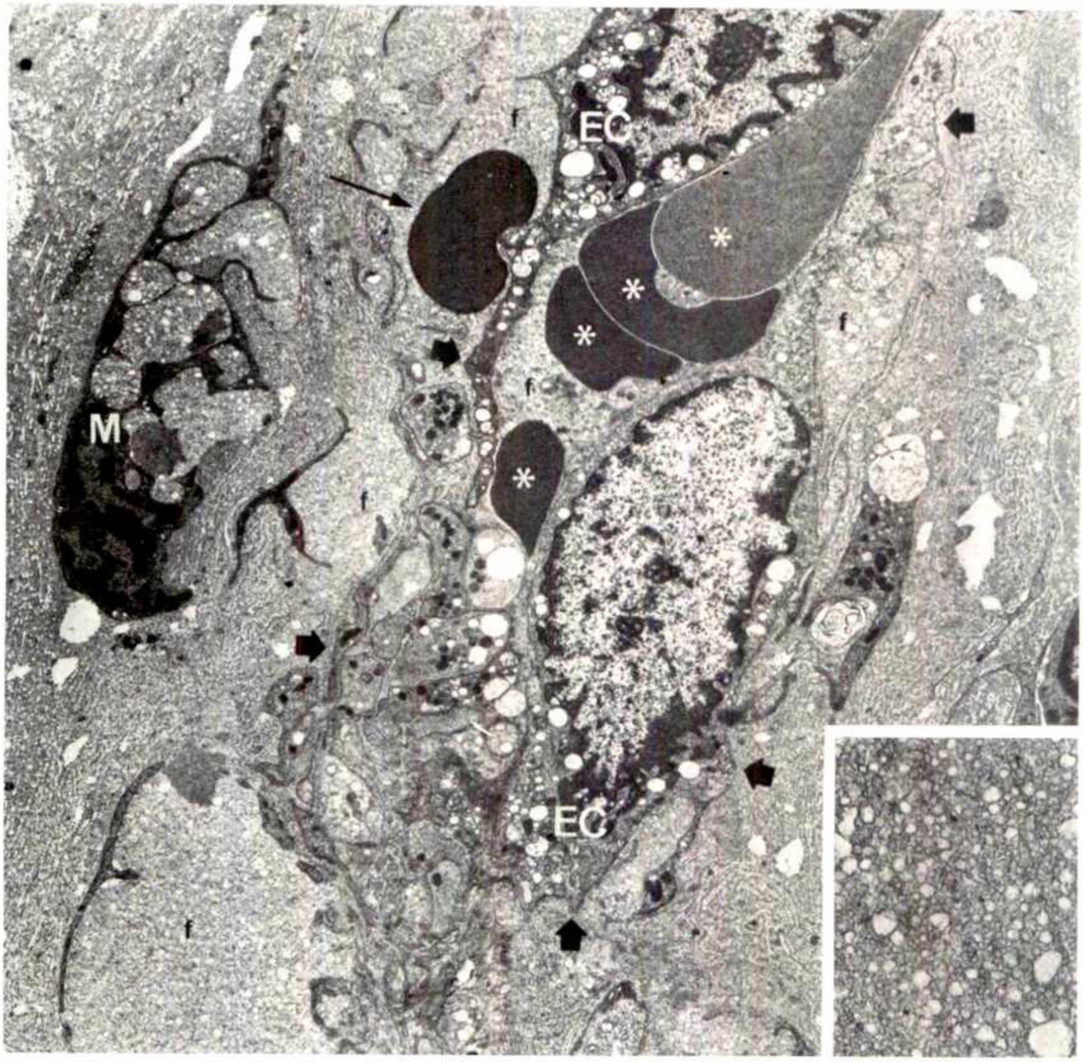

FIGURE 3. Skin of the dorsum of the hand, biopsied 2½ h after exposure to sunlight. The endothelial cells (EC) of a papillary vessel (thick arrows) are highly vacuolated, the lumen is packed with RBC (asterisks) and foamy material (f) which is also found in the perivascular tissue and is being phagocytized by a macrophage (M). The inset demonstrates a higher magnification of this foamy material. The thin arrow denotes an extravascular red blood cell (×4500; inset ×9500).

the epidermis appeared unaltered except for a focal widening of the intercellular spaces. Sixteen hours after irradiation the intercellular oedema was more pronounced and there were occasional keratinocytes that exhibited some of the known UV-induced structural alterations, such as nuclear and perinuclear oedema, irregular dense bodies, vacuoles and vacuolated keratohyalin (Nix *et al.*, 1965; Hönigsmann *et al.*, 1974). Occasional sunburn cells were present in the upper epidermal layers.

The dermis exhibited the structural changes characteristic for chronic EPP lesions as described for the non-irradiated control sites. At 2½ h after irradiation there were no other morphological alterations but 16 h after exposure there was some vasodilatation and polymorphonuclear leukocytes

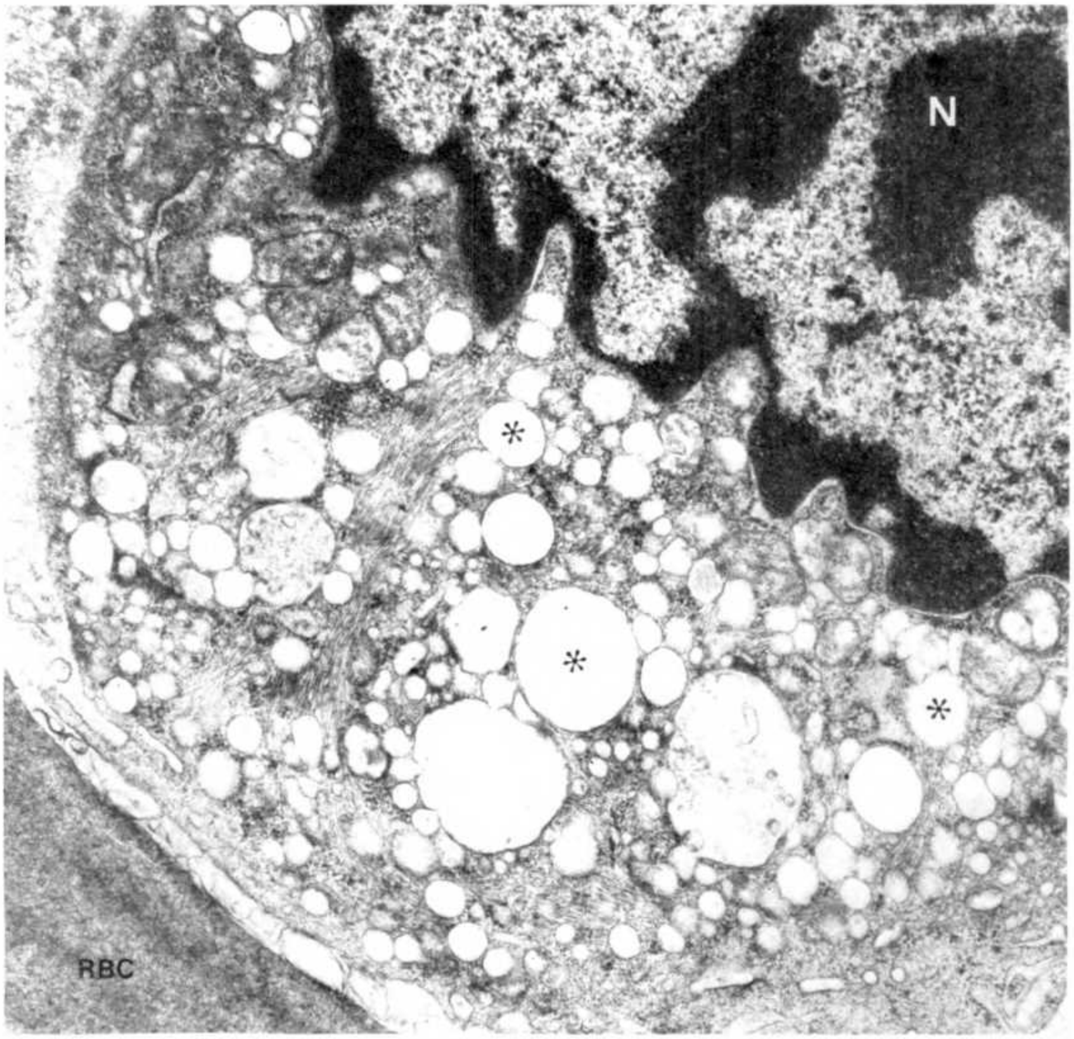

FIGURE 4. Endothelial cell $2\frac{1}{2}$ h after sunlight exposure. The cytoplasm is condensed and contains multiple vacuoles (asterisks). N, nucleus; RBC, red blood cell (× 22,200).

were seen in the perivascular tissue. The endothelial cells showed increased numbers of pinocytotic vesicles but were otherwise normal in appearance and their junctions were tight.

(b) *Reactions to UVA* (irradiation for $2\frac{3}{4}$ and 3 h, respectively, with the glass-filtered lamp). Two and a half hours after irradiation there was intercellular oedema within the epidermis; at 16 h the oedema was more pronounced and there were a few intracytoplasmic vacuoles but there were no other alterations. Sunburn cells were not noted. As after the exposure to sunlight, the most striking alterations occurred in the dermis and were again confined to the superficial vessels. Two and a half hours after irradiation there was pronounced damage of the endothelial cells, engorgement of the vessels, vascular leakage and extravasation of red blood cells (Fig. 6). These vascular changes were identical with those observed in EPP skin exposed to sunlight.

Sixteen hours after irradiation identical changes were observed within the dermis but the vascular

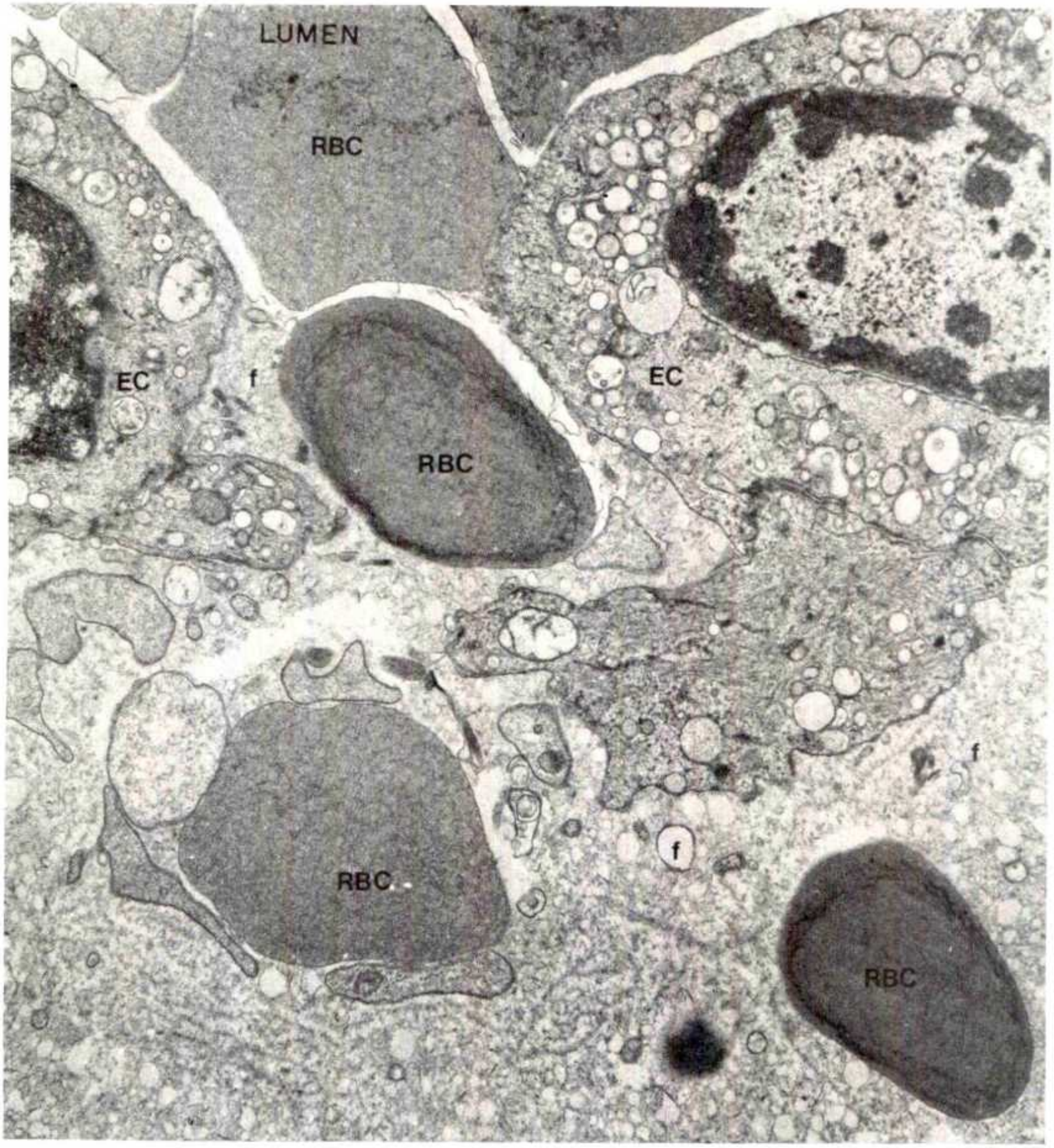

FIGURE 5. EPP skin from the dorsum of the hand biopsied 2½ h after exposure to sunlight. Two damaged endothelial cells (EC) have lost intercellular contact. Note pronounced leakage of red blood cells (RBC) and foamy exudate (f) (× 12,000).

damage was even more pronounced. In some instances, the endothelium appeared completely destroyed and cellular debris was identified within the foamy material. Other capillaries had collapsed, presenting as a cluster of necrotic endothelial cells surrounded by their multiple basal laminae (Fig. 7). However, even severely damaged vessels also contained endothelial cells that looked morphologically intact (Fig. 8) and occasionally exhibited nuclear features of cell contraction. Some of these vessels exhibited a completely disorganized structure appearing as a conglomerate of intact and lysed

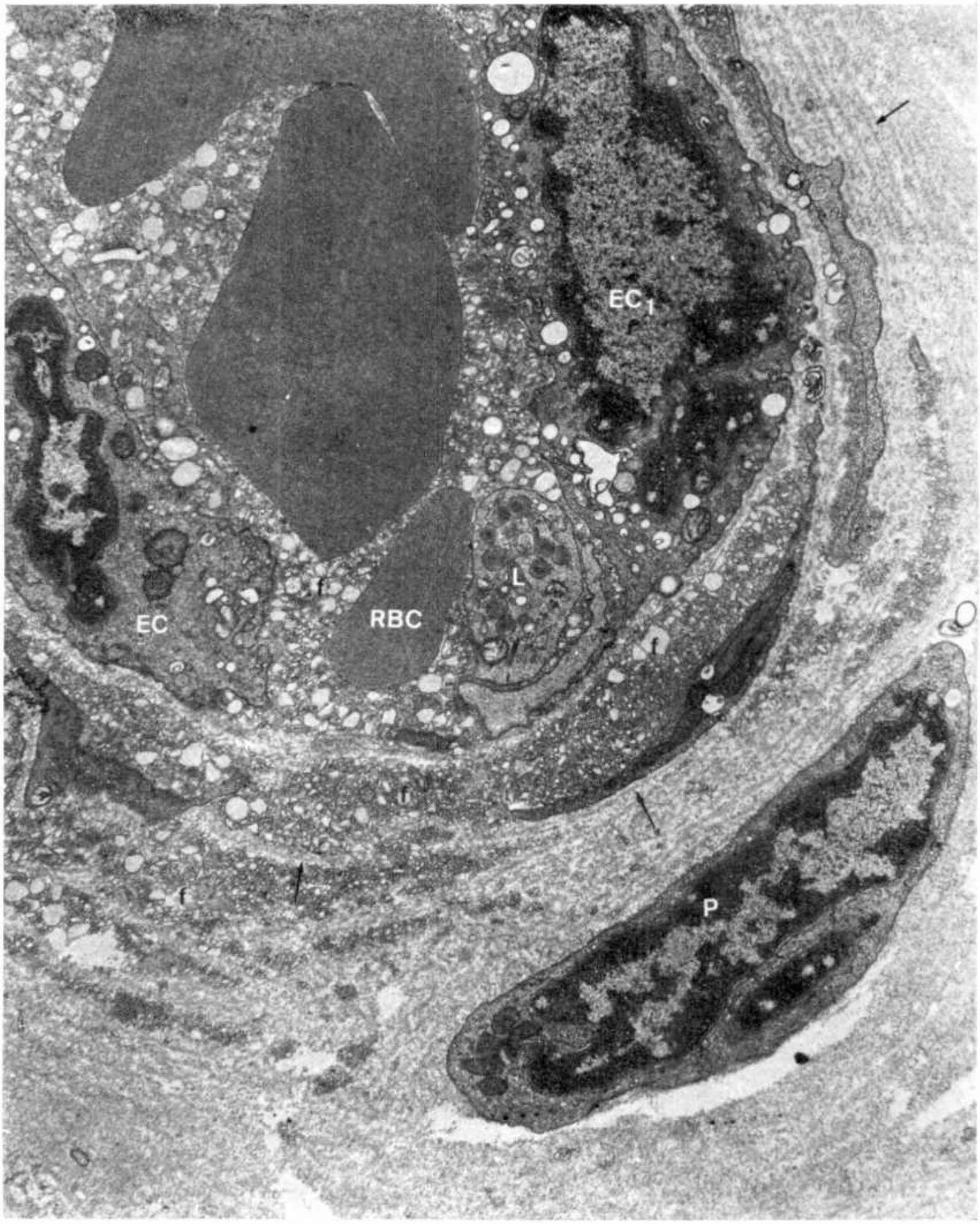

FIGURE 6. EPP skin biopsied 2½ h after long wave UVR (UVA) delivered by the artificial light source. There is a wide gap between two endothelial cells (EC) through which leakage of red blood cells (RBC) and foamy exudate (f) occurs. The foamy exudate is also found between the multilayered basement membranes (arrows). A pericyte (P) appears intact but one of the endothelial cells (EC_1) is vacuolated. L, leukocyte (× 11,000).

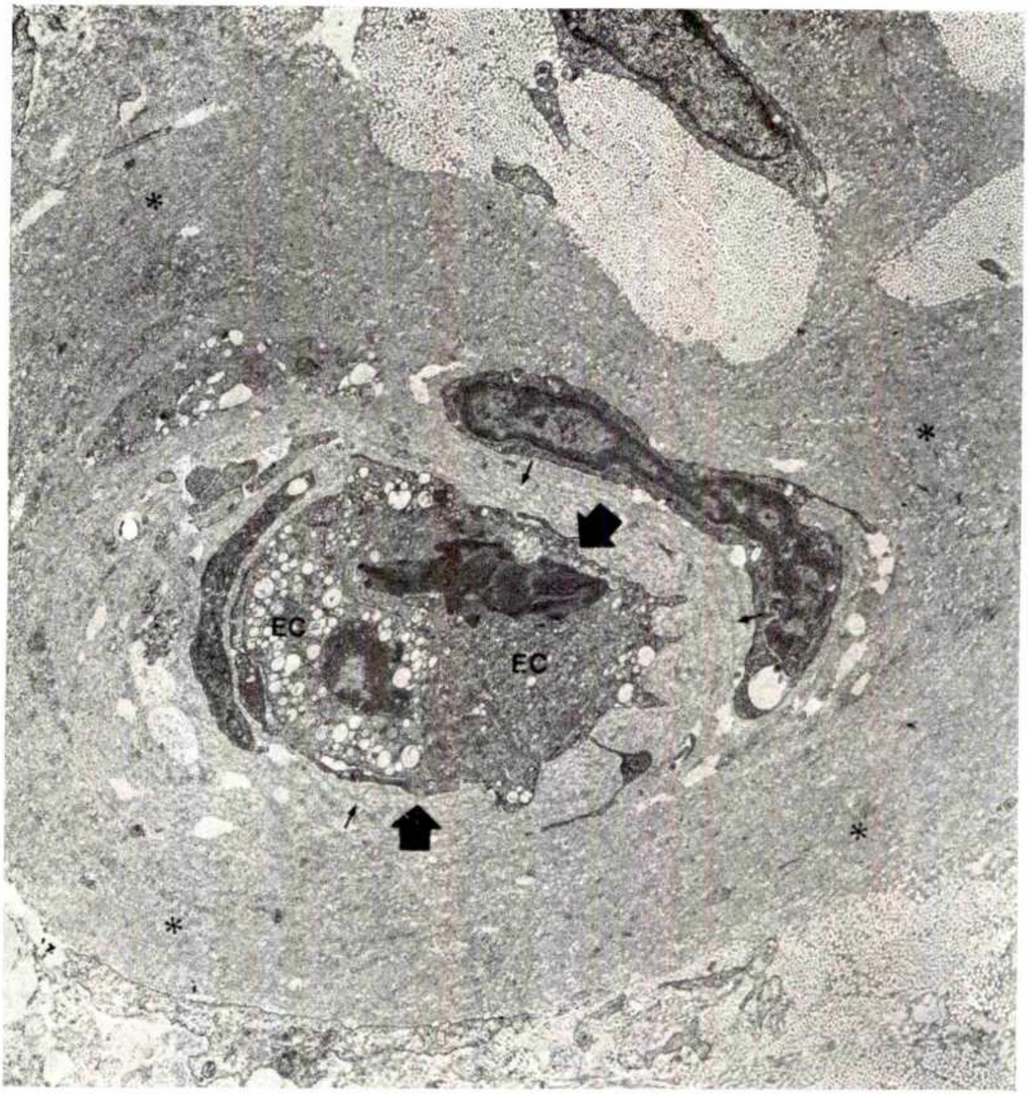

FIGURE 7. EPP skin 16 h after exposure to artificial UVA. This vessel (thick arrows) has collapsed and there is a severe degeneration of the endothelial cells (EC). Note reduplications of the basal laminae (arrows) and fibrillar material (asterisks) characteristic for exposed EPP skin (× 4500).

endothelial cells, leukocytes and red blood cells (Fig. 8). The 'foamy' material was seen to extend through wide areas of the papillary dermis and there were perivascular macrophages that had engulfed this substance. Leakage of red blood cells was pronounced and erythrophagocytosis by pericytes and macrophages was a common finding. A large number of intact and lysed polymorphonuclear leukocytes and an occasional lymphocyte were seen both within the damaged vessels and in perivascular location.

DISCUSSION

Previous studies on EPP patients have focused on the nature and derivation of the hyaline material deposited within the dermis of chronically sun-exposed skin sites. They have suggested that at least

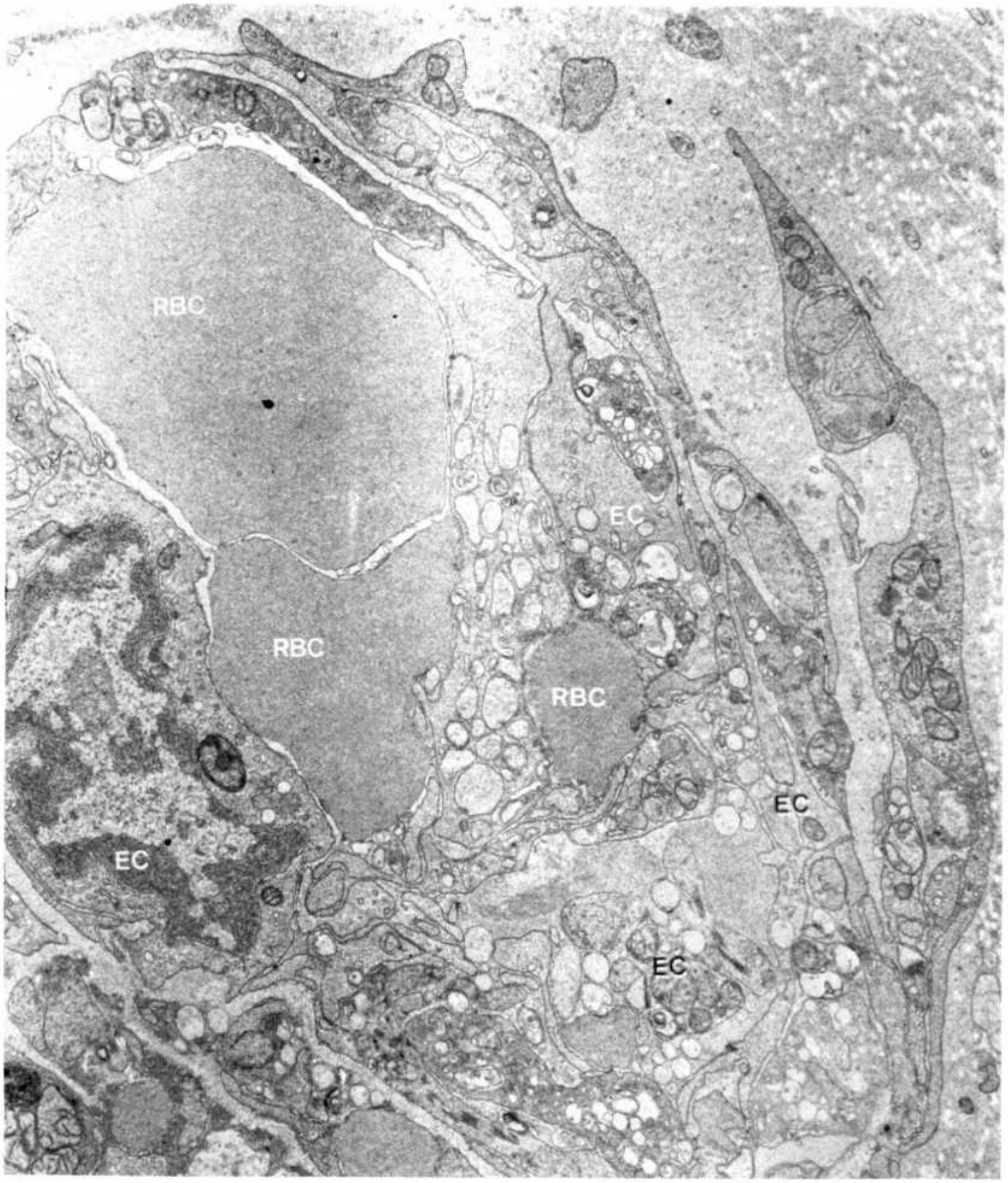

FIGURE 8. EPP skin 16 h after irradiation with artificial UVA. The wall of this blood vessel appears completely disorganized. There is a conglomerate of red blood cells (RBC) and of lysed and degenerating as well as intact endothelial cells (EC) (× 12,500).

some of this material is derived from the plasma and have thus implicated an involvement of the dermal vessels in the pathogenesis of the cutaneous lesions.

These conclusions were mainly based on the multiple concentric basal laminae around the vessels and the vascular cuffing by the fibrillar material (Anton-Lamprecht & Bersch, 1971; Ryan & Madill, 1968); on the presence of tryptophan within this material, an amino acid that is present in the plasma but absent from collagen and elastic tissue (Ryan, 1966); and on the immunohistochemical demonstration of plasma-derived globulins, fibrin, and complement within the perivascular deposits (Epstein *et al.*, 1973). Rimington *et al.* (1967) have suggested that the perivascular changes could result from (i) a direct action of the photodynamic stimulus on the vessels; (ii) its action on dermal components, i.e. mast cells or (iii) an indirect involvement of the vasculature via mediators released from the epidermis. The present study provides evidence that the vessels are indeed severely and specifically involved in the acute flare in EPP; moreover, it does suggest that the endothelial cells of the involved vessels are the sites of the photodynamic reaction.

The action spectrum in EPP is in the vicinity of 400 nm (Magnus *et al.*, 1961) and these wavelengths are known to penetrate to the level of the superficial dermis. The endothelial cells were severely damaged in all skin sites that had been irradiated with a sufficient amount of long wave UVR whereas such changes were absent from the sites exposed to erythematogenic doses of short wave (unfiltered lamp). This and the fact that endothelial damage, as described in this paper, is not known to occur in reactions due to the sunburn spectrum indicate that it was due to the long wavelengths both in the natural sunlight and in the glass-filtered artificial light source. An intriguing finding of this study is the selectivity of the endothelial damage. Endothelial cells were consistently and severely affected whereas pericytes, fibroblasts, and the epidermis appeared intact. The photosensitizing agent in this disease, protoporphyrin, is known to be present, in excessive amounts, within red blood cells (Magnus *et al.*, 1961) and to circulate within the plasma (Rimington *et al.*, 1967) but nothing is known about the porphyrin content of the vessels proper. Since the plasma protoporphyrin levels are usually low (Rimington *et al.*, 1967) one could hypothesize that the metabolite is preferentially taken up by and stored within endothelial cells to make them susceptible to long wave UVR as shown in this study. The constant bathing of endothelial cells in plasma could account for this phenomenon even in view of the low plasma protoporphyrin levels. Previous studies have shown that only minute amounts of protoporphyrin can be recovered from the epidermis (van Gog & Schothorst, 1973) and this is in accord with our observations that epidermal cells are not involved in the cellular response of the photodynamic reaction.

There is another piece of evidence supporting the selectivity of endothelial cell damage and its relative specificity for protoporphyric disease. We have recently produced identical vascular changes in protoporphyric mice irradiated with black light whereas exposure to the short UV wavelengths of a mercury lamp failed to elicit endothelial damage (Konrad *et al.*, in preparation). The latter produced the classical histological and ultrastructural features of a 'normal' sunburn reaction.

On the basis of the presented findings we are therefore proposing that the primary event of the photodynamic reaction in EPP is endothelial damage. Swelling and disruption of intracytoplasmic organelles and vacuolization of the cytoplasm characterize a process of degeneration that may proceed to cytolysis and terminate in cell death. The damaged cells fail to maintain intercellular contact, losing their capacity to seal the vascular channel, and wide intercellular gaps appear which permit an early and pronounced leakage of plasma and RBC into the extravascular compartment. This appears to be the substrate for the oedema and purpura observed clinically in this disease.

According to Cotran & Majno (1964) there are three types of reaction patterns in vascular injury.

(i) *The histamine type of leakage* which affects venules and is characterized by a partial dissociation of the endothelial cells. The endothelial cells proper remain intact.

(ii) *Direct vascular injury* manifests as endothelial damage. It is a rather drastic but nonspecific type of injury that spares no vessels. The basement membrane is usually intact, but local breaks may occur, and there may be superimposed changes of the histamine type response. Heat is one known cause of this type of injury.

(iii) *Delayed, prolonged leakage.* This leakage occurs predominantly from capillaries and tends to spread along the terminal portion of the arterioles and the root of the venules; a separation of mildly injured endothelial cells may occur in the presence of the intact basal lamina.

The described vascular damage in EPP corresponds rather precisely to the direct vascular type of injury, possibly in combination with the histamine type of response. This is well in accord with our observation of degranulated mast cells in the vicinity of the affected vessels. It should be noted, however, that apart from being degranulated the mast cells appeared undamaged and we therefore believe that mast cell degranulation was secondary to the endothelial injury.

The appearance of the 'foamy' fluid present within and leaking from the damaged vessels was unusual and unlike the exudate usually seen in inflammatory reactions. This material contained short fibrils that may represent fibrin but it was mainly composed of vesicles of varying size and shape, of an amorphous material, and of fragments of membranes that may be taken to indicate that the plasma contained residues of lytic cells. Destroyed endothelial cells are the most likely candidates to account for some of this material; although we did find some morphological evidence for intravascular and extravascular haemolysis this was only minor as compared to the endothelial damage, which is well in agreement with previous conclusions that photohaemolysis does not occur, to a significant degree, in EPP *in vivo* (Fleischer *et al.*, 1966).

At present, we can only postulate that the foamy exudate, i.e. plasma and cell debris, represents the basic substrate for the formation of the fibrillar material that characterizes the hyaline deposits in chronic EPP lesions. It appears reasonable to conclude that the multiple basement membranes that surround the dermal vessels in such lesions reflect a sequence of multiple consecutive injuries to the capillaries—as demonstrated in this study—and subsequent reparative processes. Since capillary endothelial cells build their own basement membrane (Majno, 1965) the multiple basement membranes in EPP may result from regenerating endothelium in analogy to the process of capillary basal lamina thickening in diabetes mellitus (Vracko & Benditt, 1970). Experiments are in progress, using the animal model referred to above, to test this hypothesis and to find the link between the 'foamy' exudate of the acute flare and the 'hyaline' material of the chronic lesion.

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Study of a new anomaly of the vermilion border of the lip

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SUMMARY

A new anomaly of the vermilion border of the lip is described in which part of the vermilion border is replaced by normal skin. Thirty-six cases are reported of this new anomaly, seven of which sought medical advice for this condition.

Our attention was first attracted to this condition by a 16-year-old girl who was worried about the aesthetic appearance of her lower lip. Thirty-six cases have since been observed and investigated and biopsies were carried out on three.

CASE REPORTS

Of the thirty-six patients, all were Egyptian, fifteen female and twenty-one male. Their ages ranged between 16 and 60 years with the majority between 25 and 35 years. The lesions were stationary and had not progressed after the condition was first observed in early life. In five patients there was evidence of the condition being familial. The majority of patients were non-smokers and their occupations were not mainly out of doors. Despite the unusual appearance of the lips only seven presented because of the cosmetic appearance. One of these, a young doctor, became so neurotic about it that he developed the habit of lip-biting. In contrast, another patient thought the anomaly was a sign of beauty.

The lesions, which affected the upper and lower lips equally, presented as discoloured patches of the vermilion border of the lip having the same hue and pattern as of normal skin. The lesions were well defined, neither raised nor depressed and appeared as extensions of the normal skin replacing part of the vermilion border. One or both of the lips might be involved by single or multiple lesions. The number, size and shape of the lesions varied greatly and they were classified as follows:

(1) Triangular patches of varying sizes with the base continuous with the normal skin.
(2) Quadrangular patches (Fig. 1).
(3) Band shaped patches (Fig. 2).

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(4) Irregular patches of variable sizes, single or multiple (Fig. 3).

(5) Irregularity of the lip margin giving it a fringed appearance.

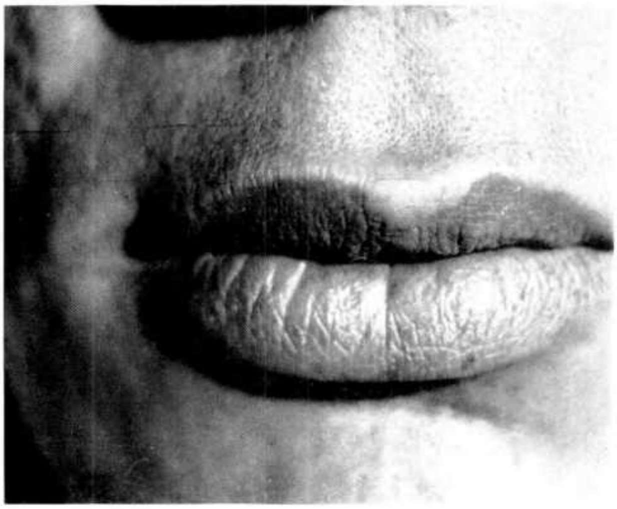

FIGURE 1

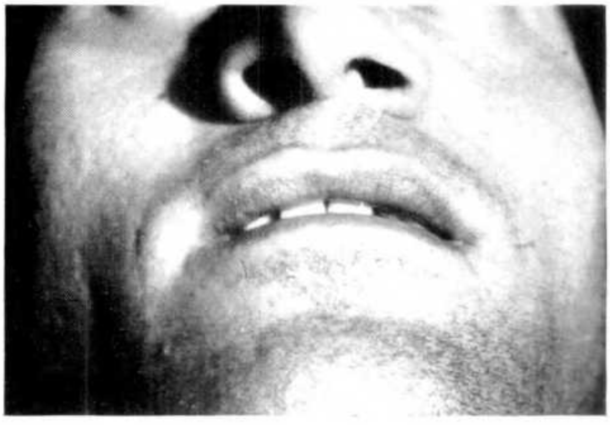

FIGURE 2

In one 17-year-old female the whole of the upper lip was affected. However, she also had marked subungual hyperkeratosis of all finger- and toe-nails with dystrophy of nail plates. She also had localized hyperkeratosis of the soles, hyperhidrosis of the face, palms and soles and papular pink lesions in the central part of the face. The mucous membranes were unaffected.

Histopathology

That part of the skin surplanting the vermilion border was normal in appearance with stratified squamous keratinizing epithelium and epidermal appendages. The adjacent vermilion surface was

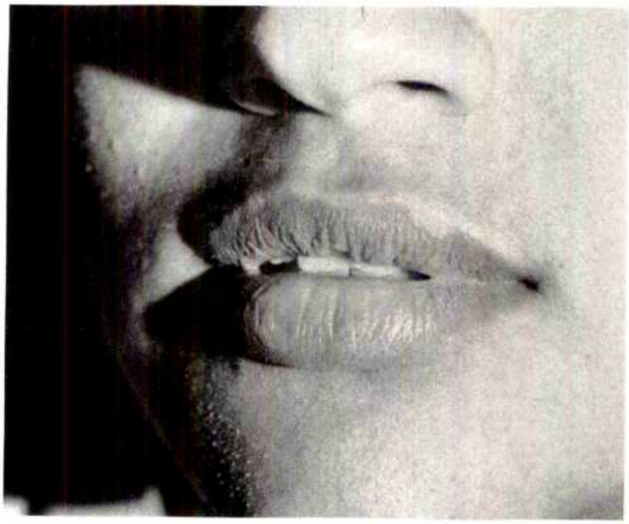

FIGURE 3

also normal non-keratinizing stratified squamous epithelium without epidermal appendages and there was no evidence of solar keratosis or any inflammatory reaction in the corium.

DISCUSSION

This new anomaly of the vermilion border of the lips would appear to be ectopic skin at the interface between the skin and the oral mucosa. It is a congenital condition with a familial tendency and may be likened to that commonest of all conditions, Fordyce spots of the lips, i.e. ectopic sebaceous glands. Recognition of this developmental anomaly is important in the differential diagnosis of similar lesions affecting the lips such as leukoplakia, vitiligo, lupus erythematosus and scleroderma. However, the presence of the lesions since childhood and their stationary nature should allow their true nature to be recognized.

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The absorption of arsenic and its relation to carcinoma

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SUMMARY

A test dose of arsenic was given to four patients with arsenical carcinoma. Blood arsenic levels, urinary and faecal excretion were measured and compared with those of three normal control subjects. Blood levels and urinary excretion were lower in the carcinoma subjects, suggesting increased storage. Abnormally high retention of arsenic, either medicinal or environmental, may be an individual metabolic trait and may be an important factor in carcinogenesis.

The mechanism by which the ingestion of arsenic leads to keratoses and carcinoma is not clearly understood, and although to some extent dose-related, it appears also to be dependent upon some individual factor.

Various authors, for different reasons, have had cause to suggest that some people are more susceptible to the carcinogenic action of arsenic than are others. Clearly it is only a proportion of those exposed to arsenic who develop carcinoma, and of these, the amount ingested, as far as can be ascertained, varies widely.

Thus, Fiertz is quoted (*British Medical Journal*, 1968) as saying that while a total dose of Fowler's Solution exceeding 400 ml provokes keratoses in about 50% of subjects, carcinoma may arise after as little as 75 ml. Fiertz's major survey (1968) is inconclusive because many subjects failed to respond to his follow-up, but it tends to point to the same conclusion and he mentions a constitutional or individual factor.

There is, also, general agreement that in patients who have arsenical carcinoma the content of arsenic in unaffected skin is higher than normal even many years after all identified arsenical ingestion has ceased.

Scott (1958) commented 'The quantity of arsenic retained in the skin bears no precise relationship to the duration of intake of arsenic nor to the time lapse which has ensued between cessation of this form of therapy and the performance of the test. This suggests that there is an individual variation in the person's ability to handle and dispose of arsenic in the body'.

Neubauer (1947) envisaged a third possibility; either there is some kind of individual reactivity to arsenic, or arsenic is not the only factor in carcinogenesis in the subjects affected.

Whether this means that carcinoma is more readily induced in some subjects by a given concentration of arsenic in the tissues, or whether some people achieve higher tissue levels after swallowing the same amount is again not known.

The skin content of arsenic in subjects who have had comparable exposure to arsenic without developing carcinoma has however not been sufficiently investigated. Scott (1958) found that, in her one patient who had no carcinoma, the skin arsenic had returned to normal 7 months after ingestion was stopped, but this was after only one month's treatment and can hardly be compared with her carcinoma patients in whom the period of arsenical treatment had averaged 24 years.

There remains, therefore, an unresolved possibility that the factor which determines carcinogenesis after arsenic ingestion is an individual difference in the absorption, storage or excretion of the substance.

An attempt has accordingly been made to follow the absorption, blood levels and excretion of a standard dose of arsenic in subjects who already have arsenical carcinomas and thus show themselves to be susceptible. These are compared with normal subjects who have never received arsenic before; the 'normal' subjects may or may not really be carcinoma-prone, but since susceptible subjects are evidently in the minority it can be assumed that most or all of the 'normal' control subjects are not in that category.

METHOD

The test subjects were patients who had been sent to the Skin Department for the treatment of arsenical keratoses and superficial carcinomas. History of arsenical treatment was established but the precise dates of this, and the total dosage could not be ascertained (Table 1).

TABLE 1.

Case No.	Sex	Age	Diagnosis	Weight (Kg)	Arsenic treatment
T 1	F	59	Epilepsy	57	Bromide & As about 15 years Skin Carcinoma 1950 onwards
T 2	M	65	Psoriasis since 1965	87	Iron & As tonic at intervals for 30 years Skin Carcinoma 1957 onwards
T 3	F	67	Epilepsy since 1926	53	Bromide & As for 5 years+ (?) Skin Carcinoma 1962
T 4	F	63	Choreo-athetosis	48	Bromide & As irregularly up to 1954 Skin Carcinoma 1960 onwards
Controls					
C 1	M	35	Psoriasis	—	None
C 2	M	66	Lichen planus 1965, 1967 Stasis ulcer	58	None
C 3	M	60	Gen. pruritus since 1961	77	None

The control subjects were routine admissions to the Skin Ward for other complaints, and in these no history suggesting arsenical ingestion could be obtained. In these patients no skin lesion suggestive of neoplasia or keratoses was present. In all the cases the patients were admitted to hospital. General medical examination showed no unexpected abnormality and liver function tests were normal.

The test subjects and the control subjects were given a standard dose of 1·25 ml of Liq. Arsenicalis B.P., equivalent to 8·52 mg As, in three divided doses at 8 h intervals on the first day. Estimation of arsenic was carried out on pooled 24 h urine daily and on 12 h whole heparinized blood for the first 2 days and thereafter daily. Faeces were collected, homogenized and analysed for arsenic. In the case of men, beard shavings were collected daily and similarly analysed.

Trace quantities of arsenic can be measured photometrically at 540 nm. Arsine is evolved by the catalytic action of zinc on reduced arsenic compounds in a hydrochloric acid concentration of about 2·2 N. The evolved arsine forms a red complex with silver diethyldithiocarbamate in pyridine. A standard curve is plotted and the unknown read off. This technique measures quantities between 1 and 10 μg (Fisons, 1967). The sample is initially treated according to the amount of organic matter it contains.

Urine

An aliquot of urine is first evaporated to near dryness. About 10 ml of concentrated nitric and 2–3 ml of 60% perchloric acid are then added and the whole boiled until 'digestion' is complete. This is signalled by the onset of a vigorous reaction with the evolution of chlorine fume and the formation of a white precipitate.

Blood

This can be left to oxidize partially in nitric acid for a few hours or overnight. A greater quantity of perchloric acid is necessary to complete this digestion.

Faeces

These are first homogenized with water and a suitable aliquot taken. The digestion is carried out in Kjeldahl flasks. After its completion the whole is transferred to small Erlenmeyer flasks and made up to about 50 ml. Two grams of potassium iodide and 2 ml of 33% stannous chloride solution in 20% hydrochloric acid are added. The arsenic is then in the reduced state ready for its evolution to arsine by catalytic zinc.

The reduction and evolution processes are carried out in specially designed glassware.*

Arsine is filtered through lead acetate wool and bubbled through 0·5% w/v solution of silver diethyldithiocarbamate in pyridine. After 45 min the optical density at 540 nm is measured. All reagents were of Analar quality; all analyses were done in duplicate, and blanks were run with each batch.

RESULTS

Blood

As expected, the highest blood levels were reached in the first 24 h, but after this daily fluctuations were considerable (Fig. 1). To some extent this may be related to excretion of arsenic into the gut and reabsorption. Broadly, blood levels were higher in the control group (Two-tail test, $P = 0{\cdot}056$).

Urine

Total daily excretion is shown in Fig. 2, and the 8–10 day totals in Fig. 3. The difference between the normal and the test group is a significant one (One-tail test, highest daily total, $P = 0{\cdot}05$; total 9 day excretion, $P = 0{\cdot}01$). The urinary concentration of arsenic in the two groups was not significantly different.

We should mention here another non-carcinoma subject who had not been added to the control group. This was a man of notably erratic habits whose daily urine volumes were low and it was suspected from the outset that his 24 h specimen was not always complete. His blood levels were as high as most control subjects and the urinary concentration of arsenic was also. A low total arsenic

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* Fisons Scientific Apparatus Ltd, Loughborough, Leicestershire.

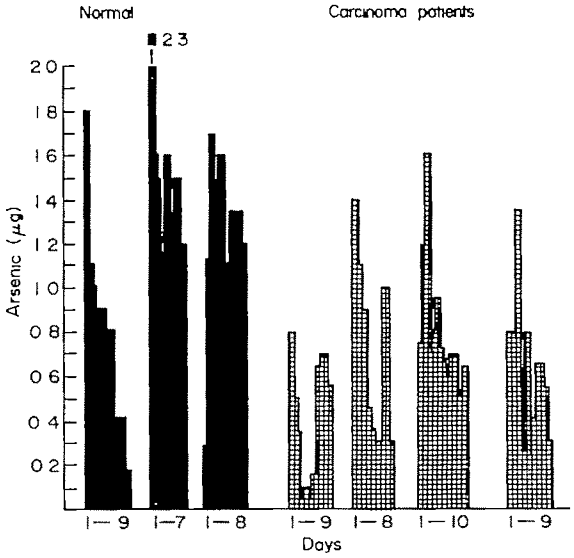

FIGURE 1. Blood levels (blood arsenic/10 ml).

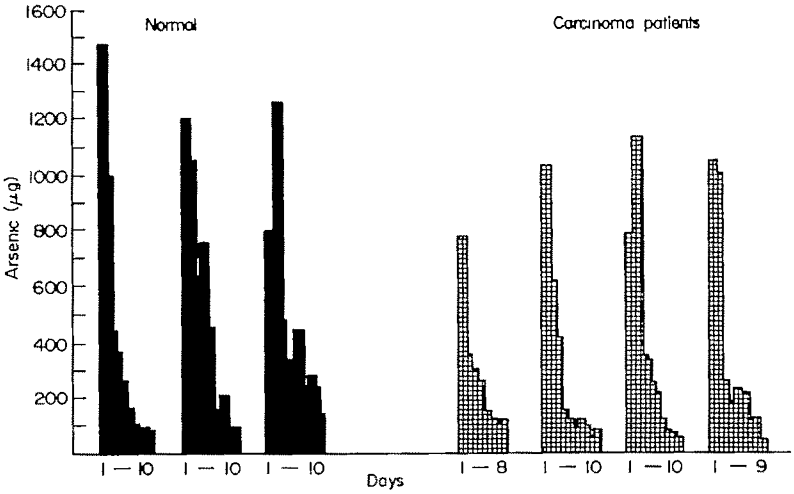

FIGURE 2. Daily urinary excretion.

value therefore accompanied low total urinary volumes, and confirmed our suspicion that some specimens were lost. Another possibility of course is that he is really one of the carcinoma-prone group who never developed carcinoma simply because he was never exposed to arsenic.

Faeces

Faecal excretion results were inconclusive. By far the major part of the dose administered was absorbed, and the total excretion over 10 days varied from 25–236 μg—at the most 3·5% of the total dose. The subject who showed the lowest figure apparently had only one bowel action in 10 days. We found no significant difference between the two groups.

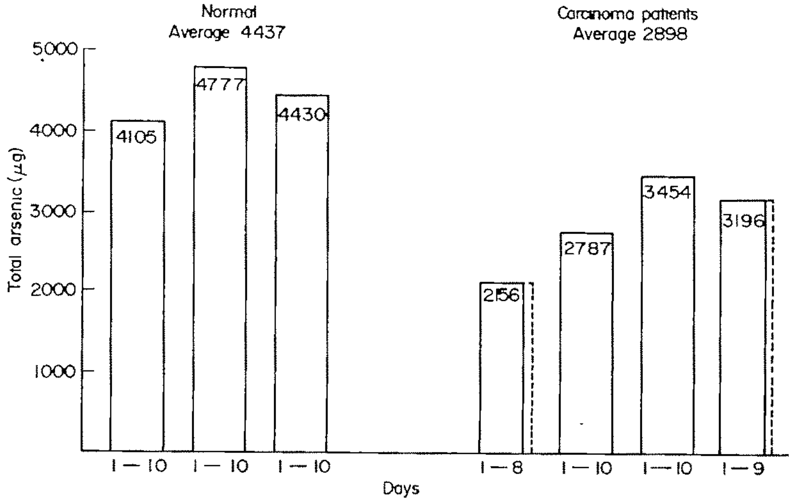

FIGURE 3. Total 10 day excretion.

Hair

Beard shavings were washed but may have been contaminated with sweat, sebum and epithelial keratin. This may explain the fact that the arsenic content showed some rise after 6–7 days. The main increase was at 10–13 days, presumably the time when the hair which was forming at the beginning of the experiment was reaching the surface. Thereafter the arsenic content fell and was usually normal or nearly so by day 20–23 when observations were concluded. We did not discern a significant difference between the test subjects and the controls.

DISCUSSION

The chief observation here is that in the carcinoma subjects blood levels were lower, and the total urinary excretion less. This is compatible with either diminished absorption or greater or more rapid removal of absorbed arsenic from the blood stream to storage tissues. The fact that the faecal levels showed no difference favours the latter.

The fate of arsenic in the human body has not been well studied. In the present series, of a total of 8·52 mg As ingested, the total amount recovered from urine and faeces reached between 2–5 mg, leaving between 3·5 and 6·5 mg unaccounted for and presumably retained in the tissues. A total of about 8–18 μg recovered from beard shavings gives no indication how much is lost through hair, nails and skin surface keratin over the whole body. However, at the extremes it seems that the carcinoma-prone subjects retain as much as twice the normal. This, rather than tissue-susceptibility to arsenic, may account for their tendency to arsenical carcinogenesis.

It is fully possible therefore that in at least some cases of arsenical carcinoma the actual quantity ingested may be small, and well below the amount which would produce effects in a normal person but that a large proportion of this is retained, so that a relatively high tissue concentration is reached in spite of a low dose. This permits the hypothesis that occult sources of arsenic, apart from medication, such as the occasional handling of weedkiller or pesticides, contaminated water or tobacco, may become relevant in highly susceptible subjects. It is, of course, well accepted that arsenic is widely distributed in nature, though in very small amounts.

One may think also of indirect industrial exposure such as among agricultural workers who are not themselves handling arsenical pesticides, but which may nevertheless come into their environment.

This may explain also the presence of above-normal amounts of arsenic in the apparently normal skin of patients who have arsenical keratoses. The persistence of arsenic in the skin so long after all known ingestion has ceased is difficult to understand. It may be that the arsenic found as much as 20 years or more after treatment is not the arsenic that was consumed and stored at that time, but results from the occult ingestion of environmental arsenic in the recent past, and which has been abnormally retained.

The part played by arsenic in the induction of Bowen's disease and other superficial epitheliomas may therefore be questioned.

The relationship between superficial arsenical carcinoma, multiple superficial epithelioma (non-arsenical) and Bowen's disease, and the differentiation of these conditions one from the other is by no means as clear for some authors as it seems to be for others. Histologically, the distinction may be difficult (Anderson, 1932; Montgomery & Waisman, 1941).

As superficial arsenical carcinoma has been shown to be associated with visceral carcinoma (Robson & Jelliffe, 1963), so has Bowen's disease (Graham & Helwig, 1959) so that there is presumably a common carcinogen. And again, as arsenical carcinoma tissue has been shown to have a raised arsenical content (Scott, 1958), so also has Bowen's disease (Graham, Mazzanti & Helwig, 1961).

One may here recall the observation of Graham *et al.* (1961) that patients with Bowen's disease showed a higher level of uninvolved skin arsenic than normal although only 5% were able to give any history pointing to possible arsenic ingestion. It may be that in those patients the amount ingested was no more than usual, only that they retained more than normal because of an individual metabolic aberration, and that this was a crucial factor in the development of Bowen's disease.

A final point; the arsenical treatment of psoriasis, though valuable, has been characterized as so dangerous as to be indefensible. It may be that this is true only in respect of a minority of arsenic-retaining carcinoma-prone subjects. If these can be identified this form of treatment may be recognized as safer, for the majority of patients, than some more modern drugs.

ACKNOWLEDGMENT

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British Journal of Dermatology (1975) **92**, 569.

Pharmacology and Treatment

Evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test

(I) HIGH AND LOW TEMPERATURE TARS AND THEIR CONSTITUENTS

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SUMMARY

'High' and 'low' temperature tars were evaluated on parakeratotic mouse tail skin, which was used as a model for the psoriatic keratinization process. The skin was examined histologically for signs of induced granular layers in previously scaly areas; epidermal thicknesses were also measured. It appears that the acidic (phenolic) fractions of coal tars induce granular layers and cause epidermal thickening, whereas neutral constituents alone only cause thickening. It is suggested that tar acids should be further investigated for anti-psoriatic activity.

Whilst investigations into the aetiology of psoriasiform diseases continue, there is a great need for safe, effective and cosmetically acceptable long-term treatments for the chronic stages of the disorders. Corticosteroids, which initially cause dramatic improvements, can produce telangiectasia and collagen atrophy when applied topically (Shuster, 1969); and prolonged therapy leading to systemic absorption, as well as parenteral administration itself, causes systemic side-effects by interference with endogenous steroids (Scroggins & Kligman, 1965; Carr & Tarnowski, 1966). Long-term treatments known to be safe and effective are coal tar and dithranol (Pegum, 1972). However, dithranol causes staining of the treated area and its use necessitates careful supervision. Coal tar preparations have been used for centuries in the treatment of many skin diseases (Kerr & Plein, 1953). Preparations containing crude coal tar are messy and smelly, but these ointments remain therapeutically superior (Champion, 1966) despite many attempts to isolate a 'therapeutically active fraction', and to improve cosmetic acceptability (Jaffrey, 1928; Obermayer & Becker, 1935; Downing & Bauer, 1948). However, many of these trials involved the screening of fractions in often poorly defined groups of patients. Hospitalized patients are well known to undergo spontaneous remissions (Samitz, 1958). Also the reports of these investigations are sadly lacking in histological evaluations of the effects of the tar fractions. Much of the variability in response of the skin to the tar preparations can be attributed to the use of a wide range of tar fractions derived from tars of similarly variable sources. The content of the tar, and hence its therapeutic nature, depends on the mode of production; the two most important parameters being the temperature of production and the type of coal used.

'Low' temperature tar is obtained at about 500°C and 'high' temperature tar is formed at temperatures in excess of 1000°C. The former has a greater number of components in lower proportions than the latter. It is unlikely that all of the hundreds of components present in coal tar are beneficial or necessary in the treatment of psoriasis. The isolation of a single component or group of compounds possessing the therapeutic activity is clearly desirable, ensuring the preparation of formulations of reproducible activity. It could lead to cosmetically clean products and to materials of enhanced activity as a result of synthetic chemical analogues based on the parent active structures.

It was decided to reinvestigate the therapeutic nature of coal tar using well standardized fractions, and employing histological evaluations of their effects. This required a model resembling human psoriatic skin. An animal model which would allow standardization by species, strain, age, sex and weight would be preferable to the use of patients for the initial large-scale screen. One such model was suggested by the work of Jarrett & Spearman (1964) who used mouse tail epidermis as a model for the psoriatic keratinization process.

Jarrett and Spearman pointed out that mouse tail-scale epidermis, unlike the rest of mouse body skin, is histologically and biochemically similar to psoriatic skin. The skin looks scaly and histological examination reveals that this horny layer is produced without the formation of a granular layer. Groups of hairs occur on the interscale regions (Spearman, 1964). It is only in areas of hair production that a granular layer appears in the mouse tail; and only here is the scaly keratin absent, and flaky 'basket-weave keratin' to be seen. The presence of a granular layer seems a prerequisite for ortho-keratinization (Jarrett & Spearman, 1964). This layer is absent in psoriatic and mouse tail skin, and both produce a more solid and parakeratotic horny layer. It has been shown that compounds, such as vitamin A, which induce a granular layer in psoriasis, can also induce this layer in mouse tail scales (Lawrence & Bern, 1958; Jarrett & Spearman, 1964). The mouse tail epidermis thus presents a very useful experimental model for screening potential anti-psoriatic tar constituents for a specific effect of granular layer induction.

MATERIALS AND METHODS

Male (Tuck's Original strain) mice of a specified weight range were treated daily with each tar ointment. Ointments were applied with cotton wool all around the tail, about 1 cm from the proximal end of the tail to about 2½ cm along its length. At the end of each run, animals were killed by stunning and dislocation of the cervical vertebrae. The tail skin was then cut along the ventral surface, stripped off and flattened for 10 minutes in 70% alcohol. The histological processing described by Jarrett & Spearman (1964) was then followed. Wax sections of 7 μ were cut and stained with haematoxylin and eosin. Each scale section was examined for the presence of a granular layer and epidermal thickness was measured with an eyepiece micrometer to include the basal layer to granular layer region. In each of ten scales per mouse, five measurements were made so that fifty measurements constituted the mean epidermal thickness for each mouse. These figures produced a group mean. Groups were compared by the students' *t*-test.

Tars and tar fractions used

The National Coal Board supplied a high-temperature tar ('Avenue' tar) and also the constituent oils and pitch separated by heating the tar to 360°C. 'Coalite' tar, a low temperature tar, was similarly provided along with its oil and pitch fractions. Both pitches were discarded, because they are brittle dense masses and are difficult to formulate. Pitch is also well known to contain a high proportion of carcinogens (Kennaway, 1924).

Tar acids. Acids were obtained by partitioning the tar and oil between 10% NaOH and a benzene/ether mixture. The aqueous alkaline phase was separated, acidified with concentrated HCl and then extracted with an ether/benzene mixture. The ethereal layer was dried (anhydrous $MgSO_4$) and the solvent was removed on a steam bath under reduced pressure.

Tar neutrals, neutrals+bases. The organic fraction left from the first separation above was shaken with 10% HCl, the protonated basic compounds being run off in the aqueous layer, leaving the neutral organic layer (1). The aqueous bases were made alkaline with concentrated NaOH. Bases were extracted with ether, dried and the solvent removed under reduced pressure. The yield of bases was very low ($<1\%$). For formulation these were combined with the neutral compounds obtained by the method outlined below.

Tar Neutrals. The remaining neutral compounds (1) were dissolved in ether and benzene, washed, dried and recovered from the solvent under reduced pressure.

Formulation

Tars and fractions were incorporated into an ointment containing 5% wool fat: 95% yellow soft paraffin. In between treatments ointments were kept at 4°C to minimize deterioration.

Experiments

Avenue and Coalite tars and oils were applied at 10% for 14 and 21 days. These were then applied along with their constituent acid, basic and neutral constituents at 40% for a period of 28 days. Controls were normal untreated mice and mice treated with the vehicle only.

RESULTS

Avenue/Coalite tars and oils

Avenue and Coalite tars and oils at 10% did not induce a granular layer in the tail scale regions. In all cases there was highly significant epidermal thickening which was similar in groups treated for 14 and 21 days (Table 1). Increased basophilia and cell hypertrophy were observed throughout the epidermis.

Acid/base+neutral fractions

After 4 days of treatment, the mice treated with 40% Avenue oil acids became hyperactive and tended to scatter when approached for a treatment, and this group was killed after 9 days as the tar was obviously irritant. The tails were darkly stained in this group after only one treatment; all mice treated with Avenue oils had very slightly stained tails.

Similar to the effect of Avenue oil acids, after two applications Coalite oil acid treated mice became hyperactive; subsequent treatment led to necrosis of the tail below the treated area and so these animals were killed after only eleven treatments. In the case of the mice treated with Coalite oils, towards the end of the treatment period of 28 days, their tails seemed to be showing signs of early necrotic changes. Skin staining by Coalite oil acids was seen after one treatment. After two treatments, Coalite oil treated mice showed signs of staining, which was not so advanced as in the acid treated group. At termination, these mouse tails were fairly patchily stained in all degrees, whereas staining by Coalite oil acids was uniform all over the treated area.

Histology

Avenue oil acids induced granular layers of varying thicknesses in only two out of nine mice. In the remainder, the skin was too eroded and in some cases, was too badly peeling to determine any such activity. Sebaceous glands were damaged. There was increased basophilia of all the epidermal cells.

Similarly Coalite oil acids produced a great deal of epidermal damage, which was so great that epidermal measurements were not possible and which prevented adequate determination of any granular layer induction.

TABLE 1. Effect of coal tar and oil on mouse epidermis

Initial no. mice/group, 10; weight range 28–32g

After 14 days	Epidermal thickness (μm)	s.e. mean	n	t	P
Control	20·6	1·08	8	—	—
10% Avenue tar	38·3	1·91	8	8·06	0·001
10% Avenue oils	30·8	0·58	7	7·93	0·001
10% Coalite tar	38·7	2·29	6	7·80	0·001
10% Coalite oils	31·2	2·98	6	3·75	0·005
Vehicle II	22·0	0·61	8	1·12	n.s.
After 21 days					
Control	20·5	1·10	10	0·04	n.s.
10% Avenue tar	34·8	0·62	9	11·70	0·001
10% Avenue oils	31·8	2·10	9	4·51	0·001
10% Coalite tar	36·7	1·54	9	8·38	0·001
10% Coalite oils	28·6	1·40	10	4·33	0·001
Vehicle II	20·2	1·14	10	0·24	n.s.

There was no significant difference between the same ointments at 14 and 21 days; Avenue tar (14) and Avenue tar (21) $t = 1·86$ (not significant).
s.e., Standard Error.

TABLE 2. Effect of coal tar and oil acid and neutral fractions on mouse epidermis

Initial no. mice/group, 10; weight range 23–27g

	Days treated	Epidermal thickness (μm)	s.e. mean	n	t	P
Control	28	23·6	1·20	7		
40% Avenue tar	28	53·2	3·09	7	8·95	0·001
40% Avenue oils (A.O.)	28	33·8	2·51	7	3·67	0·005
40% Avenue oil acids	9	40·3	1·24	9	9·54	0·001
40% A.O. Bases+Neutrals	28	31·8	2·66	7	2·81	0·05
40% A.O. Neutrals	28	37·5	1·65	8	6·68	0·001
40% Coalite tar (C.T.)	28	54·4	3·72	9	7·08	0·001
40% C.T. Acids	28	64·0	3·92	6	10·58	0·001
40% C.T. Bases+Neutrals	28	42·6	3·61	7	5·02	0·001
40% C.T. Neutrals	28	41·6	2·39	10	5·92	0·001
40% Coalite oils (C.O.)	28	40·1	1·79	10	6·97	0·001
40% C.O. Acids	11		not measurable			
40% C.O. Bases+Neutrals	28	39·3	0·96	10	10·34	0·001
40% C.O. Neutrals	21	35·1	1·40	6	6·27	0·001

In mice treated with Coalite tar acids, granular layers of one to three layers thickness were induced. In parts of the tail, the skin was eroded and peeling. Granular layers were also induced by Coalite tar, Coalite oils and in parts in a few Avenue oil treated mice. However, in the latter case, most mice suffered erosion of the skin.

Mice treated with neutral + bases and neutrals from Avenue oils, Coalite tar and Coalite oils failed to develop a granular layer. At 40%, all tars, oils and fractions caused highly significant epidermal thickening (Table 2).

DISCUSSION

There was no sign of granular layer induction until the tars and oils were applied at 40%. Although at these high concentrations there was associated extensive skin damage, granular layer induction was definitely caused by the acid (phenolic) fractions of Avenue oil and of Coalite tar and oil. This tendency was perhaps greater in the oil-acid fractions. Neutral and basic constituents and neutral compounds on their own seemed only to cause an increase in epidermal thickness with cell hypertrophy, and possibly an associated increased basophilia.

Keratinization can be altered by rubbing, which stimulates mitosis (Spearman & Garretts, 1966). However, experiments involving rubbing the tail skin daily with the cotton wool applicator showed that the rubbing when applying the preparation for about 30 s each day was not sufficient to cause the epidermal thickening (Wrench, 1973), which thus was attributed to the effects of the tars.

The epidermal thickening produced by the tars was not surprising, since tars are well known to cause epidermal thickening in animal and in human skin. In fact Gross *et al.* (1954) suggested that these properties could help protect against contact dermatitis by thickening the horny layer. However since the epidermis is already thickened in psoriatic conditions, this undesirable side-effect of the tars must be eliminated. The results of these trials with tars on the mouse tail skin indicated that the epidermal thickening effects of the tars could be reduced by omitting the neutral substances; the question of the bases cannot be settled without independent tests of the bases alone. Previous experiments with other anti-psoriatic preparations, namely a commercial 'tar' preparation 'Psorox' (Fisons Pharmaceuticals Limited), and with dithranol also produced highly significant epidermal thickening and no scale granular layers (Wrench & Britten, 1974). About 85% of the 'synthetic tar' ointment is composed of neutral substances, the remainder being acidic and basic compounds. This again shows the tendency of a predominantly neutral mixture to thicken mouse tail scale epidermis along with the failure to induce scale granular layers. Dithranol (1, 8, 9-trihydroxyanthracene) also failed to induce scale granular layers but caused very significant epidermal thickening. In their experiments with vitamin A, Jarrett & Spearman (1964) found that the epidermal thickening, produced along with granular layer induction, can be reduced with concomitant treatment with triamcinolone, probably through its anti-mitotic effect. Their combination of vitamin A and a steroid has formed the basis of a rational approach to chronic psoriatic therapy.

Skin staining by tar preparations has long been quoted as a drawback in prescribing coal tar preparations for psoriasis. Our experiments show that skin staining seems to be produced predominantly by the phenolic preparations, i.e. the only tar fraction to show therapeutic promise. This is of interest since attempts to 'clean-up' coal tar preparations in the past have led to a decrease in efficacy. Hellier & Whitefield (1967) have already suggested that the reduced potency may be due to the loss of phenols during purifications of tar involving distillation. The phenolic anti-psoriatic drug, dithranol (1, 8, 9-trihydroxyanthracene) stains the skin. Loss of the phenolic character in the triacetoxyanthracene analogue is reported to reduce staining, but there is a great loss in efficacy (Hellier & Whitefield, 1967).

Phenols have previously been reported as having a beneficial effect in psoriasiform diseases (Sulzberger & Obadia, 1956). Thorne (1963) found that a preparation containing tar acids and bases in equal proportions produced greater improvement in psoriatics than did crude coal tar.

It is suggested that the tar phenols should be further investigated to locate the specifically anti-psoriatic components.

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Evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test

(II) TAR OIL ACIDS

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SUMMARY

Mouse tail scale epidermis was used as a model to evaluate anti-parakeratotic activity of tar oil acids (phenols) boiling between 175 and 400°C. Lower boiling phenols seemed to cause more irritation, whereas high boiling phenols caused granular layer induction in formerly parakeratotic skin.

Using the parakeratotic scale regions of mouse tail skin as a model for the psoriatic keratinization process, it was found that coal tar acid and oil acid fractions induced granular layers in these scale regions where none had previously existed (Wrench & Britten, 1975). Since reinduction of a granular layer is considered to be a primary event in the healing psoriatic lesion (Van Scott & Reinertson, 1959; Komisurak, Kosek, & Schuster, 1962; Freedman, Reed & Becker, 1963; Jarrett & Spearman, 1964), it was decided to use this model further to locate a specific granular layer inducer in the acid fractions of coal tar.

MATERIALS AND METHODS

Phenolic ointments were applied daily to the proximal halves of mouse tail skin as described by Wrench & Britten (1975). At the end of each experiment, mice were killed and the tail skin was removed and subjected to routine histological processing. Wax sections of 7μ were cut and stained with haematoxylin and eosin. Epidermal thickness was measured and the epidermis was examined for the presence of a granular layer. The duration of treatments is indicated below.

Fractions used

(I) *Low boiling acids*

These were derived from 'Avenue' tar oils (a 'high-temperature' tar)

Avenue oil acids (A.O.A.)	(175–400+°C)
A.O.A. '175'	(175–185°C)
A.O.A. '195'	(195–210°C)
A.O.A. '210+'	(210–400+°C)

(II) *Individual low boiling acids*

These were derived from 'Coalite' tar oils (a 'low-temperature' tar)
Phenol, o-cresol, m/p-cresol, 2,4/2,5-xylenols
Low boiling xylenols (Lo.B.Xy.)
High boiling xylenols (Hi.B.Xy.)
High boiling tar acids (230–270°C) ('X.L.'*)
High boiling tar acids (250–305°C) ('X.X.L.'*)
*Coalite and Chemical Products Ltd.

TABLE 1. Effect of low boiling acids on mouse epidermis

Initial no. mice/group, 10; weight range 23–27g

	Days treated	Epidermal thickness (μm)	s.e. mean	*n*	*t*	*P*
Control	21	27·5	0·56	10		
5% A.O.A.	21	28·0	0·80	10	0·47	n.s.
10% A.O.A.	21	34·4	0·72	9	7·66	0·001
20% A.O.A.	12		not measurable			
5% A.O.A. '175'	21	28·8	1·08	10	1·04	n.s.
10% A.O.A. '175'	21	31·8	1·25	9	3·28	0·005
20% A.O.A. '175'	5		not measurable			
5% A.O.A. '195'	21	28·2	0·66	10	0·81	n.s.
10% A.O.A. '195'	21	29·2	1·05	10	1·38	n.s.
20% A.O.A. '195'	5	46·1	2·65	9	7·24	0·001
5% A.O.A. '210+'	21	31·7	1·07	9	3·55	0·005
10% A.O.A. '210+'	21	35·8	1·69	10	4·65	0·001
20% A.O.A. '210+'	21	48·5	3·35	10	6·18	0·001

TABLE 2. Effect of individual acids on mouse epidermis

Initial no. mice/group, 5; weight range 20–25g. Duration 14 days

	Epidermal thickness (μm)	s.e. mean	*n*	*t*	*P*
Control	28·7	1·14	7		
2% Phenol	28·7	2·14	5	0·001	n.s.
5% Phenol	31·6	1·99	4	1·41	n.s.
2% o-Cresol	28·4	1·68	5	0·17	n.s.
5% o-Cresol	29·7	2·17	5	0·43	n.s.
2% m/p-Cresol	28·2	0·99	5	0·31	n.s.
5% m/p-Cresol	35·1	2·80	5	2·38	0·05
2% 2,4/2,5-Xylenol	29·2	1·03	5	0·33	n.s.
5% 2,4/2,5-Xylenol	34·6	3·66	5	1·78	n.s.
10% Lo.B.Xy.	34·9	1·19	5	3·65	0·005
10% Hi.B.Xy.	47·6	0·82	5	12·34	0·001
10% X.L.	28·7	1·14	4	0·02	n.s.
10% X.X.L.	34·6	1·99	5	2·77	0·05

TABLE 3. Effect of high boiling acids on mouse epidermis

Initial no. mice/group, 7; weight range 20–25g

	Epidermal thickness (μm)	s.e. mean	*n*	*t*	*P*
Control	27·5	1·20	5	—	—
5% A.O.A. '200'	34·6	1·33	7	3·79	0·005
10% A.O.A. '200'	39·3	2·30	7	4·06	0·005
5% A.O.A. '225'	34·2	2·40	6	2·35	0·05
10% A.O.A. '225'	35·6	1·49	6	4·14	0·005
5% A.O.A. '290'	37·8	1·74	6	4·68	0·005
10% A.O.A. '290'	43·1	2·25	6	5·78	0·001
5% A.O.A. '330+'	32·3	1·78	7	2·07	n.s.
10% A.O.A. '330+'	45·7	2·03	7	6·96	0·001

(III) *High boiling acids*

These were derived from 'Avenue' tar oils

A.O.A. '200'	(200–240°C)
A.O.A. '225'	(225–300°C)
A.O.A. '290'	(290–330°C)
A.O.A. '330+'	(330–400°C)

Formulation

All fractions were incorporated into a base containing 5% wool fat: 95% yellow soft paraffin at the concentrations shown in Table I. This vehicle has been shown to have no effect on the morphology of mouse tail skin (Wrench, 1973).

RESULTS

(I) *Low Boiling acids*

The mice after four treatments with 20% A.O.A. became very hyperactive and were killed after a total of twelve treatments. After three treatments with 20% '175' the mice were hyperactive and after five treatments, two had died and two were showing signs of necrosis in the distal halves of their tails below the treated area, so these animals were killed; the 20% A.O.A. '195' group appeared normal, but were also killed to compare with '175' at the same concentration.

Histology. With 20% A.O.A., treated tails were impossible to measure as the epidermis was of such varying thickness. Scale granular layers were induced. In some tails the epidermis was peeling off. There was some damage to sebaceous glands. In 10% and 5% A.O.A. groups the tails appeared almost normal, sebaceous glands were intact and there were only slight increases in basophilia. However significant epidermal thickening was caused by 10% A.O.A.

The 20% A.O.A. '175' caused shedding of the whole epidermis in some cases, with hardly any keratinocyte destruction and nuclei were most obvious, although a little faded. Some scale granular layers were observed in parts of the peeling epidermis. Extensive peeling in many tails prevented epidermal measurements. The thickness of the naturally occurring interscale granular layer (Spearman, 1964) was increased. The 10% and 5% preparations produced no such effects, but 10% '175' caused highly significant thickening (Table 1).

The 20% A.O.A. '195' caused granular layer induction and peeling in six out of nine mice. However damage to the epidermis was not nearly so extreme as in the corresponding '175' group. In the remaining three mice, there were hair follicle granular layer extensions (Jarrett & Spearman, 1964) and increase in the thickness of the interscale granular layers. The 10% and 5% ointments did not cause any such changes in morphology.

Four out of ten mice treated with 20% A.O.A. '210+' had granular layers induced all along the length of the tail, and in the remaining six, there were slight extensions of the hair follicle granular layer. This latter observation was also made in 10% A.O.A. '210+' treated mice, whilst the 5% preparation was without effect. All three concentrations produced highly significant thickening of the epidermis.

(II) *Individual low boiling acids*

All individual phenols and xylenols failed to induce granular layers at the concentrations used. There were slight hair follicle granular layer extensions in the 10% X.L. and X.X.L. treated groups. Significant epidermal thickening was produced only by 2% and 5% m/p-cresol, 10% Lo.B.Xy., Hi.B.Xy. and X.X.L. (Table 2).

(III) *High boiling acids*

There was slight staining of the skin in mice treated with 10% A.O.A. '200' and '225'.

Histology. The 5% A.O.A. '200' treated mice tails were normal. At 10% this preparation induced a few scale granular layers in only three mice, and these were not continuous along the length of the tail.

The 10% A.O.A. '225' caused similar changes to the '200' ointment but at 5% there was some evidence of hair follicle granular layer extensions. These observations were also made after treatment with 5% and 10% A.O.A. '330+'.

The outstanding preparation was 10% A.O.A. '290'. At 5% there were a few isolated granulated cells just beneath the horny layer, but at 10% granular layers had been induced which were about one cell thick.

All preparations except 5% A.O.A. '330+' produced epidermal thickening as shown in Table 3.

DISCUSSION

Previous work has shown that mouse tail skin is an excellent model for evaluating agents which directly induce a granular layer in psoriatic skin (Jarrett & Spearman, 1964, Spearman & Jarrett, 1974; Wrench & Britten, 1975). Wrench & Britten (1975) found that granular layer induction in mouse tail scales by coal tar was only produced by the phenolic fractions. The above experiments with Avenue tar oil acids, notably the phenol and cresol mixtures (175–185° and 195–210°C) and the higher boiling tar acids (210–400°), suggested that relatively more granular layer induction was occurring with relatively less epidermal thickening by treatment with higher boiling fractions, although these differences were very small. However, in runs with 20% preparations rich in phenol, it was obvious that the lower boiling fractions were very much more irritant. This was the first suggestion that the higher boiling acids may be more beneficial.

The second indication came from work on Coalite tar oil acid fractions, where the individual low boiling phenols, cresols and xylenols were without effect, except to produce significant thickening with the meta- and para-cresol mixtures and also with the high-boiling xylenols and high-boiling tar oil acid mixtures. The slight extensions of the hair follicle granular layers by the latter two fractions also suggested that the higher boiling acids should be examined.

Finally the tests with high boiling acids derived from Avenue tar oils boiling between 200–400°C, seemed to highlight greater activity particularly in the range 290–330°C, with lesser activity by phenols boiling over 330°C and between 225–300°C and virtually none by the acids boiling below 225°C.

The acids boiling in the ranges 225–300°C and 290–330°C are extracted from 'Creosote' and 'Anthracene' oils respectively. These fractions contain many isomers of substituted polyhydroxyphenols. From their work with dithranol (1,8,9-trihydroxanthracene), Hellier & Whitefield (1967) suggested that such phenols may contribute to the therapeutic action of coal tar in psoriasis.

Results from these experiments suggest that a more specific and effective treatment for the chronic phases of psoriasis could be developed using these high boiling phenols which induce granular layers in chronically parakeratotic areas. The problem of irritation seems to be reduced when the lower boiling phenols are omitted. Also the slight skin staining in these experiments was confined to mice treated with the lower boiling acids. With further screening of the components of Creosote and Anthracene Oils Acids some of the compounds may form the basis of preparations long-awaited for the long-term treatment of psoriasis. These preparations would be free of the irritant and staining properties which made the parent tars so cosmetically undesirable for the psoriatic patient.

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Bio-assay of corticosteroids for topical application

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SUMMARY

Five commercially available corticosteroid creams were compared with respect to their thinning action on mouse tail epidermis. The greatest effects were produced by Synalar and Sintisone; hydrocortisone had the least action. In addition it was shown that the epidermal thickening induced by vitamin A could be significantly reduced by Ledercort cream. The thinning action of the fluorinated steroids on the epidermis is an important property of these agents when used for the treatment of dermatoses having a hyperactive epidermis. The mouse tail test is considered to be a useful screening test for new topical applications of these substances.

Probably the most widely used method for the comparison of the various corticosteroids available for therapeutic use is the skin blanching test reported by McKenzie & Stoughton (1962). Although this is useful for determining whether or not the steroid has penetrated the skin, and for the comparison of the effective penetration of different vehicles for a particular steroid, its value in indicating whether one steroid is a more potent therapeutic agent than another is more doubtful. It is most unusual for local steroids to be given for the express purpose of causing vasoconstriction, and it was thought, therefore, that a test more relevant to the action producing the desired therapeutic response would be of greater value for comparative purposes.

Probably the two actions most commonly responsible for the cutaneous therapeutic effects of corticosteroids are inhibition of epidermal mitosis and lysosomal stabilization (Jarrett, 1973). A reduction of skin thickness is thought to reflect the former effect as earlier work (Jarrett & Spearman, 1964) correlated thinning of the epidermis with a reduction in mitotic activity. Inhibition of cell proliferation with these agents was first demonstrated by Baker & Whitaker (1948) by using ACTH. Later Green & Ghadially (1951) showed that topically applied cortisone acetate inhibited mitosis in mice, and Bullough (1952) reported that the same substance completely inhbited mitosis in cultured mouse ear epidermis in a concentration of 25 mg/ml. A similar effect was demonstrated in rat epidermis by Studer & Frey (1952). Braun Falco, Thianprasit & Klint (1963) found that fluorinated corticosteroids inhibited respiratory enzymes and suggested that this may be the reason for their superior therapeutic effects compared with the non-fluorinated steroids. Jarrett & Spearman (1964) produced evidence that topical triamcinolone reduced the epidermal thickening induced by the local application of vitamin A in both mouse and human epidermis.

In the present investigation commercially available preparations of topical corticosteroids were compared with respect to their thinning activity on mouse tail epidermis. It was thought that the information derived from this type of study would be of more interest to the practising clinician than the more scientific approach of examining the different compounds in a standard vehicle. These applications are available for the treatment of skin disorders, and it was therefore thought that the thinning effects on the epidermis could be directly related to their potential therapeutic effectiveness for dermatoses in which a reduction of mitotic activity is a desirable feature.

MATERIALS AND METHODS

The various preparations were applied daily to the tail skin of male mice of the 'to' strain for a period of 3 weeks. Each mouse weighed approximately 30 g; after killing with chloroform the dorsal tail skin, about 3 cm from the base of the tail, was removed for histological examination. The tissue was fixed in 70% ethanol, and sagittal sections were prepared and stained with haematoxylin and eosin.

Epidermal thickness measurements were made by means of an eyepiece graticule from the dermo-epidermal junction to the base of the horny layer. The mouse tail is particularly suitable for this purpose as the dermo-epidermal junction is virtually flat and runs parallel to the surface. Ten different measurements were made on each tail scale, and ten scale regions were examined from each tail. This gave a total of 100 readings from each tail; the arithmetical mean was calculated for each mouse, and the statistical mean was determined for the number of mice in each group.

The mean together with the standard deviations were calculated for each group, and the *t*-test was used to compare each group of animals with the control group. All the steroids, including hydrocortisone, caused significant thinning of the epidermis.

The following commercially available corticosteroids were evaluated: triamcinolone acetonide 0·1% cream (Ledercort cream), betamethasone valerate 0·1% cream (Betnovate cream), hydrocortisone 1·0% cream, fluocinolone acetonide 0·025% cream (Synalar cream) and prednisolone stearoylglycolate 1·9% cream (Sintisone cream). In addition the effect was examined of topical 0·1% triamcinolone cream on reducing the epidermal thickening induced by the local application of water solubilized vitamin A acetate in a dose of 25,000 units per gram.

TABLE 1. Reduction in epidermal thickness after treatment with the corticosteroids

Corticosteroid	Epidermal thickness (μm) Mean	s.d.	Number of animals (*n*)
Controls	26·1	±1·5	10
Hydrocortisone cream (1%)	20·6	±2·8	6
Betnovate cream (0·1%)	18·2	±1·8	10
Ledercort cream (0·1%)	17·1	±3·4	10
Synalar cream (0·025%)	14·9	±4·0	10
Sintisone cream (1·9%)	13·4	±1·9	5
Vitamin A acetate 25,000 units/g	96·1	±11·8	10
Vitamin A acetate 25,000 units/g *plus* Ledercort cream (0·1%)	43·0	±7·9	10

RESULTS

The percentage thinning of the epidermis after treatment with the various steroids is shown in Fig. 1. Fig. 2 shows the reduction of the induced thickening of the epidermis by vitamin A when Ledercort cream is added to the mixture.

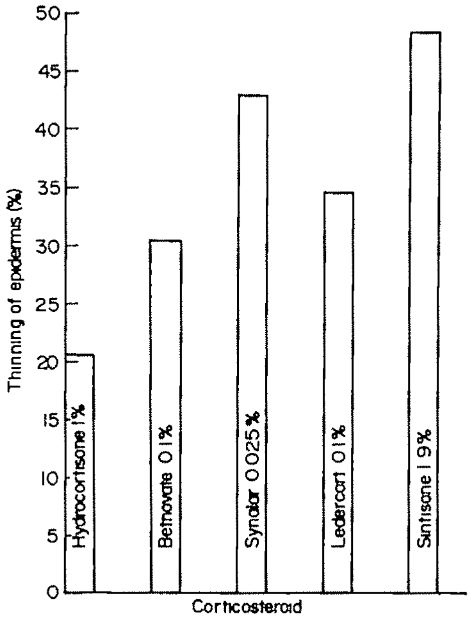

FIGURE 1. Percentage thinning of mouse-tail epidermis.

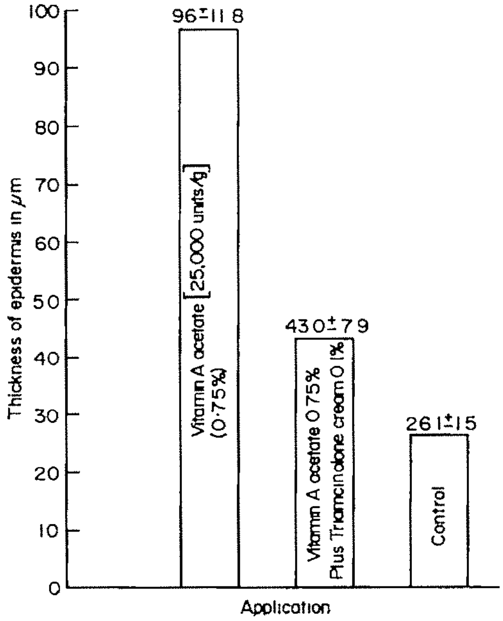

FIGURE 2. Reduction of induced vitamin A thickening with Ledercort cream.

DISCUSSION

All the topical corticosteroids tested caused significant thinning of the epidermis, and this has previously been related to their ability to inhibit mitotic activity of the epidermis (Jarrett & Spearman, 1964). This is further illustrated with respect to the action of triamcinolone cream in greatly reducing the increased mitotic activity induced by the application of water solubilized vitamin A acetate.

It is thought that the thinning effect of the various preparations closely follows the clinical response obtained when these are used for the treatment of proliferating epidermal disorders. Thus hydrocortisone has little if any effect on psoriasis, and this might be expected from the fact that it produced the least thinning effect. Synalar improves psoriasis when applied topically, and this was the most effective epidermal thinning agent investigated.

Although the greatest thinning was produced by Sintisone cream this was present in a far greater concentration than any of the other steroids; it was almost eight times more concentrated than Synalar cream, but the difference between the two groups was not significant.

The mouse tail bio-assay technique appears to be an efficient screening test for topical corticosteroids. In addition it has the advantage that changes in the type of keratin formed in the tail scale region can be detected by using the thioflavine T—congo red fluorochroming technique (Jarrett & Spearman, 1964). This may be of help in suggesting whether or not a particular application might be of use for treatment of psoriasis. It should be made clear, however, that other bio-assay methods will have to be used to assess other actions of corticosteroids. These include their suppression of cell mediated immunity, and their direct action on lysosomal membranes.

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A new formulation of 0·1% hydrocortisone cream with vasoconstrictor activity and clinical effectiveness

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SUMMARY

A new formulation of hydrocortisone with vasoconstrictor activity has been shown to be effective clinically and was more effective in a 0·1% concentration than 1·0% Hydrocortisone Cream BPC. Its use should obviate the adverse effects of fluorinated steroids.

Fluorinated steroids have deservedly achieved a prime position in the management of steroid responsive dermatoses. Recent work (Wilson, Williams & Marsh, 1973; Munro & Clift, 1973) suggests that the fears of pituitary-adrenal suppression may have been exaggerated, but every practising dermatologist is conscious of an increasing incidence of adverse local reactions with these preparations. Atrophy, with remarkable friability of the skin (Epstein *et al.*, 1963), worsening of rosacea (Sneddon, 1969), suppression of the signs of fungus infections (Ive & Marks, 1968), and a major role in the aetiology of perioral dermatitis (Sneddon, 1972; Weber, 1972), represent the debit side of these powerful therapeutic agents. The extraordinary rapidity with which fluorinated steroids produce clinical and histological changes in various dermatoses suggests that a quest for more potent anti-inflammatory activity with parallel adverse local effects may represent a search for what is theoretically possible as opposed to what is therapeutically necessary or desirable.

In most cases, the naturally occurring hydrocortisone is not associated with these adverse effects (Sneddon, 1974) and it might be advantageous if absorption could be increased by incorporation into a suitable base. Some solvents such as tetrahydrofurfuryl alcohol (Sarkany *et al.*, 1965) and dimethyl sulphoxide (Coldman, Kalinovsky & Poulsen, 1971) will actively enhance the absorption of hydrocortisone and fluocinonide respectively, but these solvents are not regarded as acceptable ingredients for widespread topical application.

Recently, attempts have been made to improve the absorption of hydrocortisone by hydrating the skin with urea (Floden *et al.*, 1971; Almeyda & Burt, 1974), but these formulations are still inferior to the fluorinated steroid preparations when judged on a corresponding concentration basis; and they do not appear to produce pallor when tested by the vasoconstrictor assay (McKenzie & Stoughton, 1962). In addition, derivatives of hydrocortisone, e.g. hydrocortisone butyrate (Polano & Kanaar, 1973) have been prepared for the treatment of eczema which, although not containing halogen, are synthetic substances and are not metabolised by normal enzymatic degradation because of esterification of the 17-hydroxyl group (Greaves, 1971).

In order to obtain an acceptable level of therapeutic activity when hydrocortisone is applied to the skin, it is necessary to reduce the particle size. This is confirmed in the specification for Hydrocortisone Ointment BP and Hydrocortisone Cream BPC which limit the particle size of the hydrocortisone. It would obviously be much more convenient to dissolve the hydrocortisone in a suitable solvent because, by this simple process, the minimum particle size possible, i.e. molecular, can be obtained. However, under these circumstances, there is a surprisingly pronounced diminution in activity since pharmaceutically acceptable solvents bond with the hydrocortisone and prevent it being partitioned into the skin. A solution system which would allow the hydrocortisone to be released might provide improved clinical efficacy.

The method adopted was to add to the solvent system an aqueous solution of a surface active agent, producing a two phase system which interferes sufficiently with the partition characteristics of hydrocortisone to mobilize it onto the substrate (in this case the skin). Both cationic and anionic surface active agents were tried and the formulations were examined by the McKenzie/Stoughton assay for physiological activity. The system which produced maximum activity was a formulation (Dioderm) based on propylene glycol and a critical proportion of an aqueous solution of sodium lauryl sulphate. There is no urea or other keratolytic agent present.

Vasoconstriction studies

Dioderm was applied to the skin under polythene occlusion and the minimum concentration of hydrocortisone required to produce consistent vasoconstriction in most patients was found to be 0·1%. This strength cream was compared with 1·0% Hydrocortisone Cream BPC and with a placebo base corresponding to Dioderm by the vasoconstrictor assay. Equal quantities of the creams were applied to a standard sized area of skin of the forearm and the sites were occluded for 16 h. The polythene film was removed and the arm examined for vasoconstriction after the effects of hydration had worn off. The results were as tabulated in Table 1.

TABLE 1. Vasoconstrictor results

Formulation	No. of patients tested	Vasoconstrictor responses
Cream containing 0·1% hydrocortisone dissolved in propylene glycol (Dioderm)	10	9
1·0% Hydrocortisone Cream BPC	10	1
Placebo base corresponding to Dioderm	10	0

These results demonstrated vasoconstriction in normal skin using a formulation containing a low concentration of the naturally occurring corticosteroid hydrocortisone. Up till now, only creams containing the synthetic and halogenated corticosteroids have been known to produce significant vasoconstriction, and as far as we know Dioderm is the only natural hydrocortisone preparation which behaves in this way.

It was evident that the suggested formulation of hydrocortisone might represent a safer way of treating steroid responsive dermatoses (with the possible exception of psoriasis which, for special reasons only seems to respond to the halogenated corticosteroids), and a series of clinical trials were instituted to investigate its clinical efficacy.

Clinical trials

In the first half of the trial fifty-two patients with a wide variety of different types of eczema were treated with Dioderm (21 patients) or with Dioderm C, which contains in addition 1·0% clioquinol (31 patients). Patients with psoriasis were not included. The results with the two preparations were similar.

A good response or complete clearing was achieved in thirty-three patients, some improvement in nine and no improvement in nine. One patient did not attend for follow-up.

In the second part of the trial, Dioderm was used in a paired comparison with 1·0% Hydrocortisone Cream BPC in patients with eczema who had bilateral lesions of similar extent and severity. The results were read by the clinician who was unaware of the identity of the preparation. In twenty patients, Dioderm was superior to 1·0% Hydrocortisone Cream BPC, two showed equal improvement on both sides and seven failed to respond to either preparation.

In addition, three patients with rosacea were treated, obviously an insufficient number for conclusions to be drawn, but two of these responded so well that a larger scale trial of rosacea patients is being organized.

The results of the clinical trials were submitted to an independent statistician for evaluation.

(a) *First half of the trial:* The clinical effectiveness of both Dioderm and Dioderm C is confirmed to be statistically highly significant (at a level of 5% or less).

(b) *Second half of the trial:* The observed superiority of Dioderm over 1·0% Hydrocortisone Cream BPC is confirmed to be statistically highly significant (at a level of 3·5%).

CONCLUSIONS

It has been shown that a topical application of hydrocortisone (Dioderm) is capable of producing vasoconstriction in the concentration and formulation in which it is used. Further, Dioderm is clinically effective in the treatment of steroid responsive dermatoses and is significantly more therapeutically active than previously described formulations of hydrocortisone. This clinical activity correlates with the ability of the formulation to produce vasoconstriction at 0·1%, the concentration employed in the test preparation.

As Dioderm contains a relatively low concentration of hydrocortisone, it would appear to be a very suitable preparation for prolonged use or where relatively large areas of skin are involved and in children.

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Mr C.Brill, Fellow of the Institute of Actuaries, carried out the statistical analysis.

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Case Reports

Graft versus host reaction and lichen planus

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The occurrence of a lichen planus like eruption during a graft versus host reaction (GVHR) has not been reported previously.

In April 1973 we observed a patient undergoing a severe GVHR (grade IV) 22 days after being treated for post-hepatitis aplastic anaemia by a histocompatible bone marrow transplantation from his sister. The cutaneous as well as liver and intestinal lesions improved within 1 month and the patient entered the quiescent stage of GVHR, according to Slavin & Santos' (1973) criteria, without severe cutaneous sequelae. On day 102 after grafting, he presented mucous membrane and cutaneous lesions typical of lichen planus. Buccal and nail lesions were of the erosive variety. Histological examination of a palmar lesion confirmed lichen planus. The liver and small bowel remained unaffected. The cutaneous lesions improved very slowly over 3 months leading to a poikilodermatous state. One year later, the cutaneous sequelae are extremely severe, with dermal sclerosis, epidermal atrophy, cicatricial alopecia, reticulate pigmentation, hyperkeratosis, and widespread ulceration showing no tendency to healing. The skin complications are now life threatening in spite of a very good haematological result and of the absence of liver or bowel disease.

Histological similarities between GVHR and lichen planus have been described by Slavin and Santos; focal necrosis of the epithelial basal layer and dermal infiltration by lymphocytes are prominent features of both diseases. However, the lymphocytic infiltrate is usually sparse in early cutaneous GVHR and the clinical aspect of GVHR has never been reported to be lichenoid.

Our case report seems to be important for two reasons: it is the first clinically and histologically verified lichen planus occurring after a severe GVHR has healed. This association does not seem to be fortuitous, and a second case is reported in this issue (Saurat *et al.*, 1975). The scarcity of such cases can be explained by the usually short survival of patients with severe GVHR.

This lichen planus was followed by severe skin changes. Such lesions have occurred previously after severe GVHR (Storb, personal communication). Maybe lichen planus following GVHR was the initial event.

The significance of lichen planus in our cases is not obvious. It could be a persisting chronic GVHR exclusively located on the skin: lichen planus following GVHR would be a model of cell mediated autoimmune disease.

Another hypothesis is that a lichenoid eruption, whatever its background, is a way of cellular reaction (immune or not) to a necrosis (from various aetiology) of the basal cells, as suggested by Pinkus (1973) and Black (1972). These hypotheses are not mutually exclusive. Dermatologists have to pay attention to chronic GVHR. Further research into cutaneous GVHR could give a new insight into the pathogenesis of lichen planus.

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Graft versus host reaction and lichen planus-like eruption in man

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Lichen planus-like eruptions can be seen in various circumstances, but graft versus host reactions (GVHR) occurring in bone marrow transplanted patients have never been reported as an aetiological factor. The sequence of histological events in lichen planus (LP) seems to include initial epidermal basal cell damage (Pinkus, 1973). The mechanism of this damage is unknown (Black, 1972). Skin lesions of GVHR in man appear to be related mainly to basal cell damage (Slavin & Santos 1973). Because of the similarity of the initial lesions in LP and in GVHR, lichenoid eruptions in GVHR could be expected. Nevertheless, GVHR skin eruptions have never been reported to be clinically lichenoid; maculo-papular or dry scaling scarlatiniform rashes and even toxic epidermal necrolysis (Peck, Herzig & Elias, 1972) have been noted in the acute stage of GVHR, or atrophy and pigmentation in the 'quiescent phase' (Slavin & Santos, 1973). Slavin & Santos (1973) first drew attention to the similarity between the histology of lichen planus and of GVHR; this similarity was observed mainly during a secondary phase of GVHR called by these authors: 'florid phase of aggressor lymphocytes destructive lesion'.

The following is the history of a patient whose acute GVHR skin eruption was initially maculo-papular and subsequently became chronic with clinical and histological features of an extensive lichen planus-like eruption. This 13-year-old boy was admitted for post-hepatitis aplastic anaemia. Transplantation was performed with the marrow of his matched sister. On day 7, the first signs of engraftment were observed and the immediate course was uneventful. On day 11, he developed a diffuse maculo-papular rash. Two days later, high spiking fever, diarrhoea and a rise of SGOT appeared. A skin biopsy on day 11 showed a grade II GVHR without any lymphoid infiltrate. From day 18 to day 52 he received a total dose of 280 mg IgG/kg of anti-thymocyte globulin. The SGOT was normal by day 62, and diarrhoea and anorexia disappeared on day 75. The skin lesions persisted with scaling and pigmentation. A second skin biopsy, on day 56, showed persisting grade II-III GVHR, only sparse lymphoid cells were seen. On day 59, a cytomegalovirus (CMV) interstitial pneumonitis with viraemia was diagnosed. All immuno-suppressive therapy was discontinued and treatment with anti-CMV plasma, gammaglobulin and transfer factor was given. At that time there was a severe immunodeficiency (agammaglobulinaemia, unresponsiveness of lymphocytes to phytohaemagglutin or to allogeneic lymphocytes). The clinical status improved progressively but skin lesions persisted and changed. Typical papular lichen planus-like lesions appeared. They were first observed on the extremities and progressively spread to the whole body. The buccal mucous membrane and nails were also involved. A skin biopsy, on day 90, demonstrated the characteristic lesions of lichen planus: basal epidermal cell damage with colloid bodies, and a band-like mononuclear infiltrate in the upper dermis with a sharp lower margin. Direct immunofluorescence studies on the three biopsies were negative.

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The association of GVHR and a lichen planus-like eruption does not seem to be fortuitous; a second case is reported in this issue (Touraine *et al.*, 1975). Both cases had bone marrow transplants for post-hepatitis aplastic anaemia.

The interest of this association is threefold:

1. *Histogenesis.* We have observed primarily basal cell damage and secondarily mononuclear cell infiltration of the dermo-epidermal junction on repeated biopsies. The role of this infiltrate in the genesis of the epidermal damage is puzzling because lymphocytes in this patient seemed to be severely depressed. This could be a confirmation of currently accepted concepts about the histogenesis of LP.
2. *Pathogenesis.* Aetiological factors postulated in LP are numerous (Black, 1972). An auto-immune mechanism has been discussed but little supporting evidence has been forthcoming. GVHR is regarded (in part or totally) as a delayed hypersensitivity type homograft rejection (Slavin & Santos, 1973). The epidermal cell can be one of the rejected cells. Such lesions may occur in any situation (in non-transplanted patients) in which the host's own epidermal cells appear foreign to its self-recognition apparatus. This is highly hypothetical however because of the numerous non-immune factors which could be involved in GVHR. Nevertheless this hypothesis could be provocative for further research into LP.
3. *Management of bone marrow transplanted patients.* As reported in this issue (Touraine *et al.*, 1975) the lichen planus-like eruption can be followed by severe life-threatening skin lesions in the absence of haematological, liver, or bowel problems. Dermatologists must be concerned with bone marrow transplanted patients.

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Comment

COMPLEMENT DEFICIENCIES

The study of the complement system was for a long time, not without some justification, looked upon as one of the more esoteric immunological pastimes and it is only in recent years that investigations of complement function have found a substantial place in clinical practice.

From such studies has come the recognition, on the one hand, that complement component levels can be useful in the diagnosis and management of many 'immunological' diseases; and on the other hand, that there are to be found individuals with genetic deficiencies of various complement components, the study of whom can help to clarify the *in vivo* role of the complement system.

In the field of dermatology perhaps the best example of this interaction concerns hereditary angio-oedema (HAE). Although this disease has been recognized since the middle of the nineteenth century (Quincke, 1882; Osler, 1888) it was not until 1963 that Donaldson & Evans demonstrated that it was associated with a deficiency of the $C\bar{1}$-inhibitor. This serum protein, also described as $\alpha 2$ neuraminoglycoprotein, inhibits a number of serine histidine esterases found in blood plasma. These include, besides $C\bar{1}$ itself, plasmin, kallikrein, activated Hageman factor (XIIa) and activated plasma thromboplastin antecedent (XIa). HAE is a dominantly transmitted disease and affected subjects are heterozygotes having low levels of inhibitor rather than none. The characteristic episodes of angio-oedema appear to be precipitated by local exhaustion of the reduced amount of inhibitor allowing apparently autocatalytic activation of C1. This in turn leads to the wholesale activation of C4 and C2 with the generation of a kinin-like substance which appears to be the principal mediator of the disease. It is not clearly known what initiates the attack. The only certain predisposing factor is trauma. However, it seems likely that the activation of any of the enzyme systems that can consume the inhibitor can give rise to local exhaustion and spark off an attack. What brings an attack to a close 48–72 h later is also not understood but it is known that administration of inhibitor systemically can rapidly bring attacks to an end (Pickering *et al.*, 1969). The inhibitor is usually administered in the form of fresh frozen plasma but since this contains further substrates for activated C1 it would be preferable to give purified inhibitor and a source of this is likely to become available in the near future. Furthermore, prophylactic treatment of HAE is possible using epsilon amino caproic acid, or its analogue tranexamic acid, which inhibits the activation of the whole spectrum of plasma enzymes with which the $C\bar{1}$ inhibitor reacts. An increasing number of trials with this treatment have been made since it was first introduced by Lundh *et al.* (1968) and there seems little doubt that for most patients it is effective.

This is therefore a disease where both prophylactic and therapeutic treatment have been developed as a direct result of an increased understanding of pathogenesis. Since there is now treatment available the diagnosis of HAE is no longer only of academic interest. In most cases, dermatologists make the diagnosis clinically without laboratory help but levels of $C\bar{1}$ inhibitor can readily be measured by esterolytic techniques and this allows the diagnosis to be confirmed.

Other primary deficiencies of complement may also be associated with diseases with skin manifestations. Moncada *et al.* (1972) have described a family with a deficiency of C1r where the affected members suffer from repeated infection or from a disease resembling systemic lupus erythematosus accompanied by somewhat unusual skin lesions but without the characteristic serological abnormalities of SLE.

A single patient with C4 deficiency and a similar LE-like disease has also been described (Hauptmann, Grosshans & Heid, 1975). The commonest complement component deficiency in man, however, is that of C2. Here, the majority of the patients are without clinical disease but the incidence of genuine serologically proven systemic LE and of glomerulonephritis is much commoner than is to be anticipated from chance alone. A single patient with C2 deficiency has been described who has an almost lifelong history of Henoch-Schönlein purpura (Sussman *et al.*, 1973) and a further example of this association is known (Rosen, 1974). Although the mechanism by which deficiency of the early complement components leads to an increased incidence of the diseases is not certainly known, it seems not improbable that some sort of immunity deficiency is involved. In the case of C1r deficiency bacterial infections have been encountered but these have not been seen in C4 deficiency and C2 deficiency and it is more likely that there is an increased tendency to persistent infections with viruses and perhaps mycoplasma (Sussman *et al.*, 1973; Peters & Lachmann, 1974).

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Correspondence

Brief aus Graz

The German Dermatological Society meets every 3 years. It consists not only of dermatologists from Western Germany, but also from Austria and German speaking Switzerland. From a membership of over 2000, approximately 1000, together with their wives and husbands, produced a gathering in Graz of the proportions of an international congress. It was with great pleasure that a band of British dermatologists, led by Arthur Rook, accepted the kind invitation of the President, Professor W. Schneider of Tübingen, to come to Graz as guests of the German Dermatological Society.

Graz, which has seen much of the brilliance of Mozart, and was the home town of Hebra is clustered in a valley on the river Mühr. Graz provided an old city for exploration, nearby mountains, an open air museum, as well as a host of castles and churches. The ladies' programme used these to the full with a host of excursions. Nor was the programme of tours, excursions and visits intended for 'ladies only', for many of the congress members could be found dodging an academic session, which they found less captivating, and ascending a mountain or sitting at a pavement café in the sunshine. The weather throughout was warm and sunny.

Speeches of welcome referred to the visit of German dermatologists to Britain 2 years ago, and we were made to feel very much at home.

To commemorate the Congress a postage stamp with a portrait of Hebra upon it was printed. Graz lies to the east of Austria, and is quite near the Czechoslovakian border. It is gaining a reputation as a Congress Town and is connected to Vienna, by a fast local diesel electric train, over the mountains.

The local secretary was Professor Weitgasser and the congress chairman was Professor Kresbach, both from Graz. The congress lasted from registration on the evening of Tuesday, 10 until Saturday, 14 September.

The town Concert Hall provided the venue, both for lesser gatherings and the main theme meetings.

The congress opened with speeches of welcome from the president, the local chairman, the mayor of Graz, and such speeches were interspersed with some magnificent chamber music between each speech, performed by a very polished group of medical musicians. We witnessed the presentation of medals to two internationally famous dermatologists, well known friends of British dermatology. The Marchionini Gold Medal was given to Hans Götz for his services to international dermatology, and the Herxheimer Medal was presented to Felix Sagher for his work in leishmaniasis. We were delighted to be there and to add our congratulations to the acclamation of the whole meeting. Dr Rook, Dr Beare, Dr Bettley, Dr Garretts, Dr Hodgson, Dr Jarrett, Dr Pegum, Dr Rowell, and Dr Sanderson made up the British party.

The main themes of the congress comprised:

Dermatological therapy. Immunology in dermatology. Virus infections. Electron-microscopy. Genital infections. Dermatological teaching. Reticuloses of the skin.

A very well organized demonstration of clinical dermatology was presented, and the patients were seen in small alcoves within a spacious hall.

Some patients in the wards were shown on closed circuit television, using Eidophor colour television, produced by Ciba-Geigy. The colour quality was remarkably good, and the images projected on the screen were about five times life size. A sound link permitted spectators in the audience to talk to the presenters, and to Professors Korting, Schuppli and Tappeiner who discussed the cases.

A large exhibition was open throughout the congress in which drug firms, instrument makers, and booksellers demonstrated their latest products. It was nice to see a collection of old dermatological text books offered for sale and very quickly sold. Electron microscopes, phase contrast, projection ultraviolet light fluorescence instruments and photomicrograph apparatus were all on display. The standard of the papers was very high indeed, and the presenters spoke slowly and clearly. The whole meeting was an unqualified success.

We are fortunate that we do not have to wait 3 years before meeting old friends again, but a meeting such as the one in Graz is well worth waiting for.

MAURICE GARRETTS

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DERMATITIS HERPETIFORMIS, IODINE COMPOUNDS AND THYROTOXICOSIS

SIR, The well presented case report of dermatitis herpetiformis (DH) provoked by iodine, by Drs From & Thomsen (1974), has prompted us to look carefully at our own series and we find that we have four patients with a somewhat similar background. Since the recorded cases are few we would like to give brief details of these patients with particular reference to the treatment of the thyroid condition in relation to that for DH.

Case 1. Mrs M.N. aged 60 years. In November 1958 she was treated with ^{131}I for thyrotoxicosis, leading subsequently to hypothyroidism. She was maintained with 0·3 mg sodium thyroxine daily until January 1965. In December 1964 an eruption which had been present for over 1 year was diagnosed as DH. This was confirmed by histopathological examination but no immunofluorescent examination has since been done. Enteropathy was not proved but at one time she was noted to have a low serum folate. Howell–Jolly bodies were found in the blood but she was not shown to have hyposplenism.

Control of the DH was achieved by 200 mg dapsone daily for 4 days. The maintenance dose was 100 mg daily. In January 1965 she was thought to be myxoedematous again and the dose of thyroxine was raised to 0·5 mg daily for the next 6 months. The maintenance dose of dapsone went up to 200 mg daily in March 1965 and to 300 mg daily in May 1965. In July 1965 thyroxine dosage was reduced to 0·4 mg daily and in September 1965 to 0 3 mg daily. The maintenance dose of dapsone was reduced in July 1965 to 200 mg at which level it remained until May 1966, when it began to need an increase again. By 1970 the skin lesions had become out of control even with a tentative dose of 400 mg dapsone, which was deemed too risky to continue. Intravenous heparin therapy, although producing a severe anaphylactic shock reaction necessitating its withdrawal after 48 hours, resulted in return of the maintenance dose of dapsone to 200 mg daily. She is now maintained on this dose plus a gluten-free diet.

Case 2. Mr A.B. aged 70 years. Thyrotoxicosis was diagnosed in August 1966 and controlled by 50 mg potassium perchlorate daily. Early in 1968 it relapsed and was not controlled even by 800 mg of potassium perchlorate daily. This dose resulted in 'quite a bad skin condition' which persisted. Thyrotoxicosis was controlled by 300 mg carbimazole daily reducing to 15 mg daily. As he became euthyroid his skin improved but in September 1968 he was diagnosed as having DH and given 200 mg dapsone daily. This suppressed the DH lesions. At the same time he was given ^{131}I therapeutically and a further dose in 1969. In April 1969 he was doubtfully thyrotoxic and dapsone was reduced to 150 mg daily and 2 months later to 100 mg daily. By this time he was possibly euthyroid. His dapsone requirement fell slowly over the next 2½ years to between 50 and 75 mg daily. In January 1972 he was found to be hypothyroid with a low serum thyroxine. Dapsone requirements rose to 100 to 150 mg daily. He was started on 0·1 mg thyroxine daily in November increasing gradually to 0·2 mg daily by September 1973, when he again showed mild symptoms of hyperthyroidism. By this time the dapsone maintenance level had risen to 200 mg daily. He was found recently to have pyloric stenosis and its relief increased his appetite. His dapsone maintenance dose which had been 100 mg daily post-operatively rose again to 150 mg daily.

Case 3. Mrs L.McK. aged 70 years. This patient was found to have 'idiopathic steatorrhoea' with megaloblastic anaemia in 1956. In 1958 she became thyrotoxic and was treated with ^{131}I. In 1962 she became hypothyroid and thyroxine treatment was commenced. She developed a rash in May 1964 which was diagnosed as DH by a dermatologist in September 1966 and confirmed histologically in 1967.

At this period she was receiving 0·15 mg thyroxine daily and 50 mg dapsone controlled her DH. In 1971 the respective doses were 0·3 mg thyroxine and 100 mg dapsone. In September 1972 on the same dose of thyroxine she was noted to be thyrotoxic and the dapsone requirement had risen to 200 mg daily. Reduction of thyroxine to 0·2 mg daily resulted in a euthyroid state and a corresponding lower dapsone requirement of 100 mg daily. In August 1973 thyroxine 0·3 mg daily was again necessary but she became thyrotoxic and the dose of dapsone rose to 200 mg daily. In September 1973 she was found to be iron deficient with gastro-intestinal symptoms. She was put on a gluten-free diet, thyroxine was reduced to 0·2 mg daily and dapsone to 100 mg daily. She has felt well since.

Case 4. Mrs M.W. aged 36. Diagnosed as thyrotoxic in 1968, she developed an itching rash when given Lugol's iodine prior to thyroidectomy in 1968. This cleared post-operatively but recurred 2 years later and was diagnosed as DH. She was given sulphapyridine. She remained euthyroid. Her thyroxine dosage, sulphapyridine or dapsone requirements and the state of her DH subsequently were as follows:

Date	Thyroxine (mg)	Sulphapyridine (g)	Dapsone (mg)	Result
7.11.72	0·1	1·5	—	DH controlled. Premenstrual flare
28.11.72	0·1	1·0	—	DH controlled. Premenstrual flare
12.12.72	0·1	1·5	—	DH controlled. Premenstrual flare
23. 1.73	0·15	1·5	—	DH uncontrolled. On oral contraceptive
12. 4.73	0·15	—	100	DH controlled
5. 6.73	0·15	—	75	DH controlled 50 mg no control
25. 9.73	0·15	—	100	DH controlled

These four patients all had thyrotoxicosis and developed DH subsequently. In two (cases 1 and 3) the onset of DH was associated with the administration of thyroxine for ^{131}I induced hypothyroidism. One patient developed DH during potassium perchlorate treatment of the thyrotoxic state and in one patient the initial eruption was provoked by Lugol's iodine, recovered post-operatively but recurred 2 years later when on thyroxine. All four patients showed a greater or less degree of interdependence between the dose of thyroxine and that of dapsone (or sulphapyridine) required to control the DH. This was most marked in cases 1 and 3. It was also notable in these two patients that the activity of their DH was ultimately diminished by the introduction of a gluten-free diet.

Several authors from du Castel (1912) onwards have commented on the relationship between DH and thyrotoxicosis and between DH and thyroid antibodies (Fraser, 1970). This relationship has varied. DH was improved or cleared by treating thyrotoxicosis with potassium iodide (Rattner, Rodin & Barsky 1950), or by sub-total thyroidectomy (Smith 1966). According to Peck (1948) it has responded to thyroid medication if myxoedema was present. Klauder (1933) reported improvement with thyroid but did not mention the thyroid status. Hansen (1937) and Rostenberg, Fretzin & Dimmick (1966) found that thyroid medication aggravated DH, whilst Wise's patient (1933) developed DH 3 weeks after thyroidectomy. The patients of Goldin & Wilkinson (1973) and of Kumar (1973) did not appear to be influenced by treatment of thyrotoxicosis, even though in the former such treatment resulted in pretibial myxoedema and a euthyroid state.

The cutaneous response to gluten intake which results in DH is adversely affected not only by gluten but also by iodine compounds. The exclusion of either may reduce or abolish the skin lesions. Gluten-free diet does this for the majority of DH patients but the exclusion of iodine only affects certain individuals.

It is possible that the association of DH and thyrotoxicosis should be considered in terms of disordered iodine metabolism rather than just as diseases which may both be auto-immune in origin. It is known that not only is there a great increase in the incidence of HL-A8 antigen in DH (Katz *et al.*, 1972; White, *et al.*, 1972) but also in thyroid disease (Grumet *et al.*, 1973). This could be a connecting link and whilst it might be interpreted as evidence of the (auto)–immunological nature of the two diseases it could equally well be an indication of a genetically determined disordered iodine metabolism.

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Review

HAIR II

Racial and other genetic variations in hair form

Racial differences in hair form are so conspicuous that in the early stages of scientific anthropology most methods of classification depended on hair as the primary race characteristic (Haddon, 1910). As early as 1827 Bory de Saint Vincent divided mankind into the Leiotrichi, or straight-haired, and the Ulotrichi, or woolly-haired. This terminology was later adopted by Huxley, who is often wrongly stated to have devised it. In 1863 PrunerBey went so far as to say 'a single hair presenting the average form characteristic of the race might serve to define it' (Haddon, 1910). Subsequently other anthropologists proposed many different classifications, but by the fourth decade of the twentieth century other racial characters had assumed greater importance and many authorities had become dissatisfied with classifications that depended on criteria which could not be adequately quantified. Many earlier workers also had been aware that precise measurement was desirable and shaft diameter, cuticle scale count and cross-sectional index were carefully studied; the last in particular is unreliable as oblique sectioning is difficult to avoid, especially in tightly coiled hair. Other variables also were examined but no satisfactory method was devised of recording the essential differences in hair form in such a way that they could be subjected to meaningful statistical analysis.

The recent work of Daniel Hrdy (1973) of the Department of Anthropology of Harvard University is therefore a considerable advance. He selected eight variables which could be accurately measured, and which in combination, would provide a quantitative assessment of the critical features concerned in differences in hair form. The variables he measured were; (i) average diameter, (ii) medullation, (iii) scale count, (iv) kinking, (v) average curvature, (vi) ratio of maximum to minimum curvature, (vii) crimp, (viii) ratio of natural to straight length. For the definition of these terms and the techniques of measurement Hrdy's paper should be consulted: using these methods he measured hairs from seven different populations. Like previous investigators (e.g. Steggerda & Seibert, 1941) he noted the high variance within populations and within individuals, which emphasizes the importance of examining sufficiently large samples. Hrdy's findings need not be summarized in detail; of practical importance is the independence of curvature—'curl'—and crimp, and that kinking too is largely uncorrelated with curvature; these features have been hopelessly confused in the clinical literature. Medullation is largely but not completely correlated with diameter. A statistical analysis of the data supported the assumption that hair form is a polygenic trait and allowed the interesting conclusion that 'the range of genes in the population pool is limited. Interaction with the environment .... giving a wide range of expression to a single genotype'.

The fascinating syndromes in which affected Caucasoids develop 'woolly' hair, either as an hereditary defect, or as a circumscribed naevoid abnormality, have been studied by Hutchison, Cairns & Wells (1974) whose findings certainly throw much light into a dark corner of dermatology. However further progress in precisely differentiating grossly similar conditions should be possible when Hrdy's techniques are applied to the highly subjective assessments of 'woolliness', 'curliness' and 'acquired progressive kinking', which, with other equally woolly terms, abound in the scattered but not inconsiderable literature on the subject.

Attempts to relate differences in form to differences in chemical composition were made by Menkart, Wolfram & Mao (1966). They found marked differences between wool and hair, in that hair showed greater cross-linking and a higher sulphur and cystine content. However Negroid and Caucasoid hair were chemically indistinguishable. Hrdy & Baden (1973) have carried these studies a stage further. They examined hair from various populations, using the following techniques; aminoacid analysis, acrylamide gel electrophoresis, X-ray diffraction and stress–strain analysis. Identical results were obtained for all samples, suggesting that differences in hair forms are not dependent on differences in biochemical structure.

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ARTHUR ROOK

The attention of dermatologists is drawn to the recent work of Baker (1974) who writes on Race from a zoologist's point of view and is exceptionally illuminating on the subjects of pigmentation and of sweat glands.

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News and Notices

APPOINTMENTS

Surrey Area

Dr Robin Henry Felix has been appointed Consultant Dermatologist to the Surrey Area—West Surrey/North East Hants District—South West Surrey District. Dr Felix graduated, M.A., M.B., B.Chir., from Cambridge University in 1965, having trained at the Westminster Hospital. He became M.R.C.P.(U.K.) in 1971. He was Registrar in Dermatology at the Royal Marsden Hospital from 1969 to 1970 and in Medicine at St. Luke's Hospital, Guildford, from 1971 to 1972. Then he became Senior Registrar in the University Department of Dermatology, Royal Victoria Infirmary, Newcastle-upon-Tyne. Dr Felix's chief research project has been concerned with devising methods for measuring itching and measuring response to different treatments given either systemically or topically. He has also been involved in investigative work in a large series of patients who have presented with adverse cutaneous effects of Practolol.

Bath Health District of Wiltshire

Dr Robert Seng-Hoon Tan has been appointed Consultant Dermatologist to serve the Bath Health District of Wiltshire. He qualified M.B.(Cantab.) in 1965, obtained his M.R.C.P.(Lond.) in 1969 and his M.D.(Cantab.) in the same year. He trained at St. Thomas's Hospital Medical School and did his General Medicine at St. Mary's Hospital, Portsmouth and the Poole General Hospital. He was Registrar at St. Thomas' Hospital and then Senior Registrar in Dermatology at the Westminster Hospital, Westminster Children's Hospital and Queen Mary's Hospital, Roehampton. He has published a number of case reports in the British Journal of Dermatology and the Proceedings of the Royal Society of Medicine and he is interested in the Immunology of Mycosis Fungoides.

Salford Area Health Authority (Teaching)

Dr P. J. August has been appointed Consultant Dermatologist to the Salford Area Health Authority, which includes responsibility to the Manchester Skin Hospital and the Withington Hospital. Dr August qualified from St. Thomas's Hospital with M.B., B.S. in 1967 and became M.R.C.P. in 1970. He trained in Dermatology at the Liverpool Royal Infirmary and later at St. Thomas's Hospital. He has published work on 'The systemic effects of topically applied corticosteroids' and on 'Percutaneous absorption of corticosteroids'.

Stiefel Travelling Scheme

Stiefel Laboratories are arranging subsidized travel and accommodation for Dermatologists and their families to attend major international events throughout the world. Details may be obtained from Mr David Jefferis, Stiefel Laboratories, 825 Yeovil Road, Slough, Bucks.

Erratum

In the paper 'An Explanation for the "placebo" effect of bland ointment bases' by S. Tree and R. Marks (*British Journal of Dermatology*, **92,** 195), the figure 23% in line six of the summary should read 34%.

The Physiology and Pathophysiology of the Skin

Volume 3

edited by A. Jarrett

Department of Dermatology, University College Hospital Medical School, London, England

October/November 1974, xviii + 448pp., £13.00/$34.50 0.12.380603.8

With the proliferation of papers and monographs on normal and abnormal skin physiology throughout widely diversified journals of different disciplines, a need has arisen for a comprehensive survey to bring together contemporary knowledge and thinking in a form that the practising dermatologist can relate to some of his clinical problems. It is the aim of this series to fulfil this need.

Divided into two sections, the present volume concerns itself with both the dermis and the dendritic cells of the epidermis. The first section, on the dermis, gives an account of the chemistry of collagen and elastic tissues. The ageing process of the dermis is considered and the physical nature of the dermis in the living skin is discussed in some detail. Diseases affecting collagen and elastic tissues are described from the standpoint of their pathogenesis and pathophysiology, and there is also a chapter on the comparative physiology of the dermis. An account of the dermal cell population is given in the last chapter of this section.

The second part of this volume, devoted to the dendritic cells of the epidermis, considers, in particular, the melanocyte both from its biological aspects and its association with human and animal pigmentary disturbances. The relationship of the melanocyte and the Langerhans cell and the nature of malignant melanomata are also discussed.

Dermatologists, and pathologists who wish to understand something of the disordered physiology underlying the cutaneous disorders requiring their diagnosis, will welcome this work as an invaluable addition to their bookshelves. Its usefulness should also extend to biologists and all other scientists who share an interest in this field of research.

Contents

The Physiology of the Dermis

A. Jarrett: The chemistry and molecular biology of collagen.
A. Jarrett: The elastic tissue of the dermis.
A. Jarrett: The physical nature of the dermis in living skin.
R. I. C. Spearman: The comparative biology of collagenous tissues.
A. Jarrett: Ageing of the dermis.

The Pathophysiology of the Dermis

A. Jarrett: The collagenoses.
A. Jarrett: Cutaneous elastoses.
T. J. Ryan: Vasculitis.
A. Jarrett: Dermal cell populations and their pathological responses.

The Dendritic Cell Population of the Epidermis

P. A. Riley: Melanin and melanocytes.
P. A. Riley: Embryonic origin and abnormalities of melanocytes.
P. A. Riley: The biochemistry of pigment formation.
P. A. Riley: Pathological disturbances of pigmentation.
P. A. Riley: The Langerhans cell.
P. A. Riley: Melanoma.

Author Index

Subject Index

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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British Journal of Dermatology, Volume 92, Number 5, May 1975

CONTENTS

Clinical and Laboratory Investigations

Albinism in Nigeria: A Clinical and Social Study 485
A. N. OKORO

Historical Evidence for the Genetic Heterogeneity of Pseudoxanthoma Elasticum 493
F. M. POPE

An Evaluation of Na$^+$, Cl$^-$ and pH Ion-specific Electrodes in the Study of the Electrolyte Contents of Epidermal Transudate and Sweat 511
K. GRICE, H. SATTAR, T. CASEY AND H. BAKER

The Effect of the Activity of Psoriasis on the Response to Autologous Ground Skin Extract 519
COURTNEY HODGSON AND ELIZABETH HELL

Cytochemical Identification of ATPase-positive Langerhans Cells in EDTA-separated Sheets of Mouse Epidermis 5[illegible]3
I. C. MACKENZIE AND C. A. SQUIER

The Effect of Transfer Factor on the *In Vitro* Migration of Tuberculin Stimulated White Cells from Sarcoid and Normal Subjects 535
J. R. PARK, A. DE BATS AND E. L. RHODES

The Sebum Excretion Rate in Rosacea 541
J. L. BURTON, R. J. PYE, GAY MEYRICK AND SAM SHUSTER

Erythropoietic Protoporphyria—Submicroscopic Events during the Acute Photosensitivity Flare 545
F. G. SCHNAIT, K. WOLFF AND K. KONRAD

Study of a New Anomaly of the Vermilion Border of the Lip 559
ROSHDY MOHAREB, MEDHAT ELKOMY AND NASHAAT HANNA

The Absorption of Arsenic and its Relation to Carcinoma 563
F. RAY BETTLEY AND J. A. O'SHEA

Pharmacology and Treatment

Evaluation of Coal Tar Fractions for use in Psoriasiform Diseases using the Mouse Tail Test (I) High and Low Temperature Tars and their Constituents 569
ROSANNE WRENCH AND ADAM Z. BRITTEN

Evaluation of Coal Tar Fractions for use in Psoriasiform Diseases using the Mouse Tail Test (II) Tar Oil Acids 575
ROSANNE WRENCH AND ADAM Z. BRITTEN

Bio-assay of Corticosteroids for Topical Application 581
R. I. C. SPEARMAN AND A. JARRETT

A New Formulation of 0.1 % Hydrocortisone Cream with Vasoconstrictor Activity and Clinical Effectiveness 585
M. WHITEFIELD AND A. W. MCKENZIE

Case Reports

Graft Versus Host Reaction and Lichen Planus 589
R. TOURAINE, J. REVUZ, B. DREYFUS, H. ROCHANT AND P. MANNONI

Graft Versus Host Reaction and Lichen Planus-like Eruption in Man 591
J. H. SAURAT, LILIANE DIDIER-JEAN, ELIANE GLUCKMAN AND ANNETTE BUSSEL

Comment

Complement Deficiences 593
P. J. LACHMANN

Correspondence

Brief aus Graz
MAURICE GARRETTS

Dermatitis Herpetiformis, Iodine Compounds and Thyrotoxicosis
W. S. DOUGLAS AND J. O. D. ALEXANDER

Review

Hair. II. Racial and Other Genetic Variations in Hair Form 59
ARTHUR ROOK

News and Notices

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References

1. Brit. J. Derm. **92,** 585, 1975
2. Archs. Derm. **86,** 608, 1962
3. Brit. J. Derm. **90,** 197, 1974

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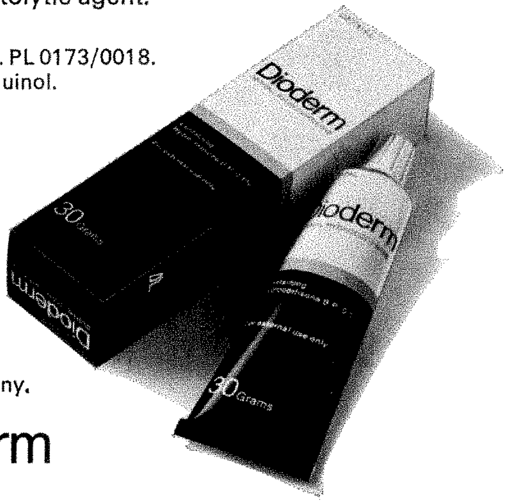

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References. 1. *Brit. J. Dermatol.*, 1968, **80**, 111; 2. *Brit. J. clin. Pract.*, 1970, **24**, 45; 3. *Int. J. clin. Pharmacol.*, 1973, **8**, 258; 4. *Brit. J. Dermatol.*, 1967, **79**, 487; 5. *Brit. J. Dermatol.*, 1968, **80**, 124; 6. *Clin. Trials J.*, 1973, **10**, 26.

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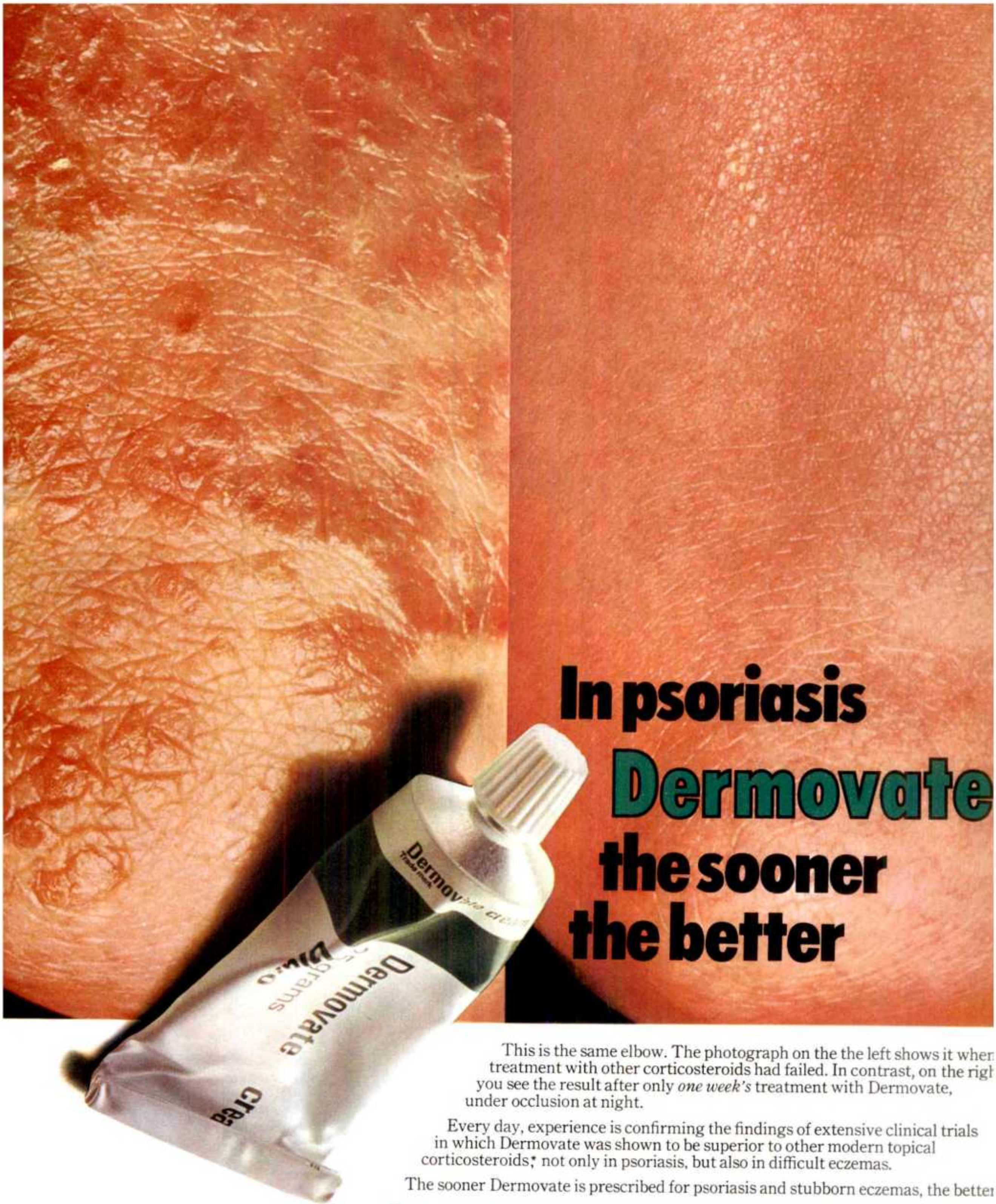

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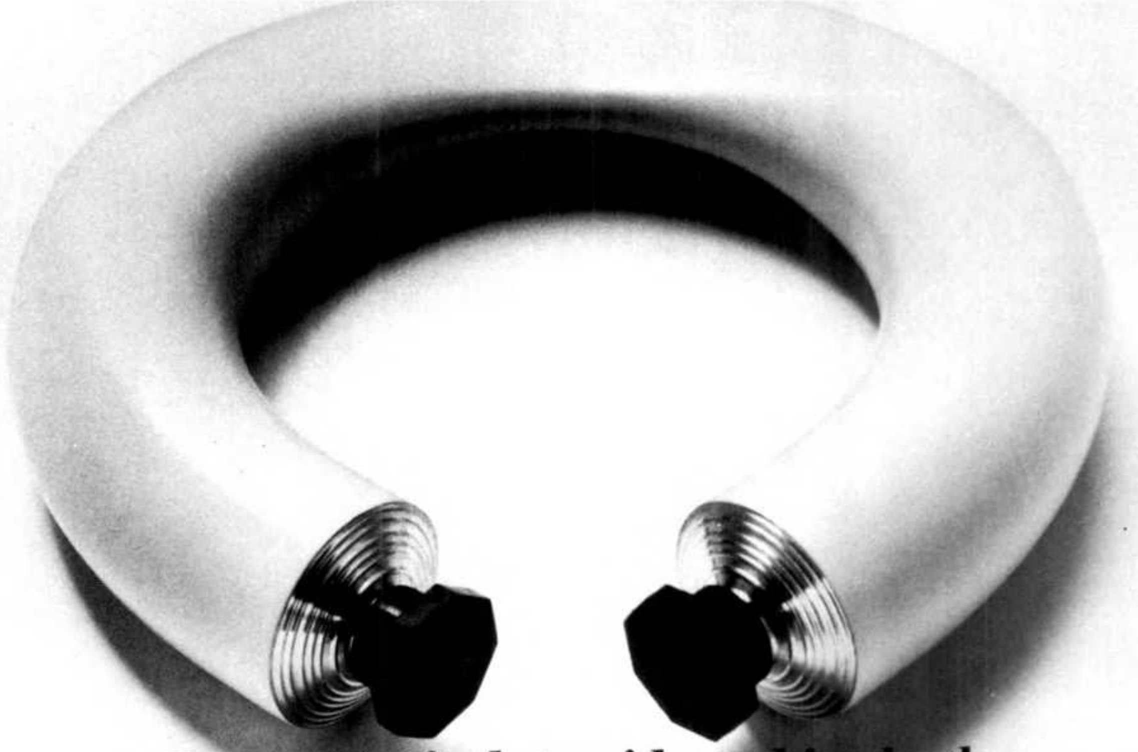

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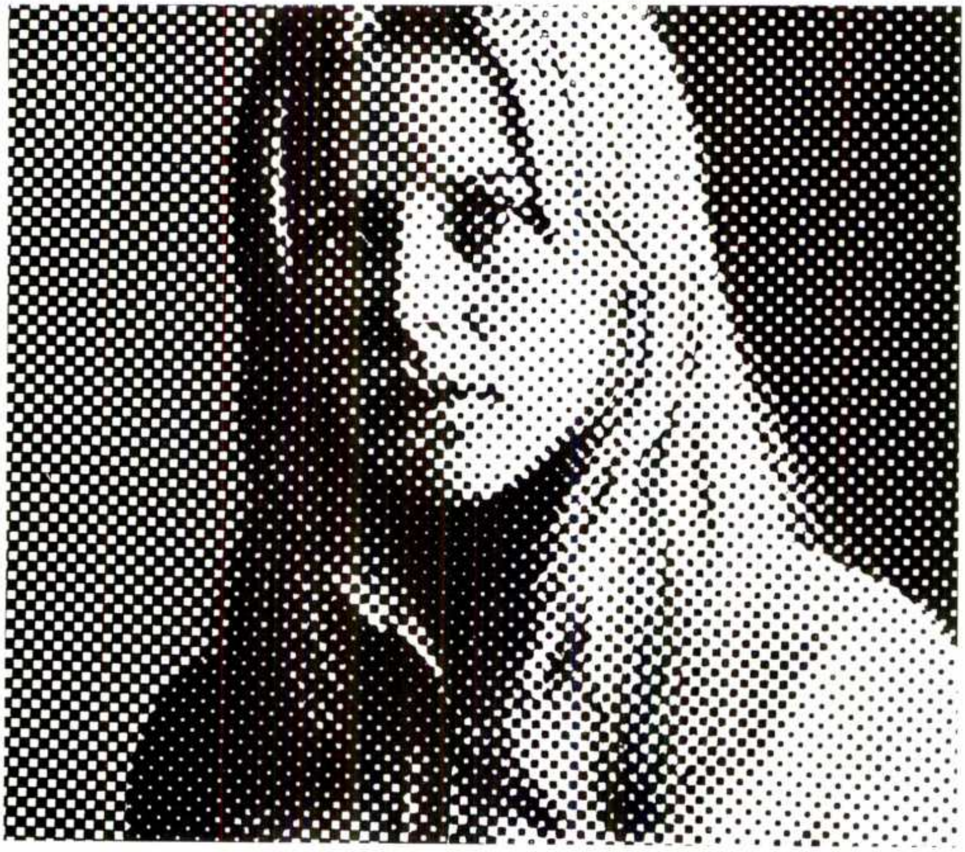

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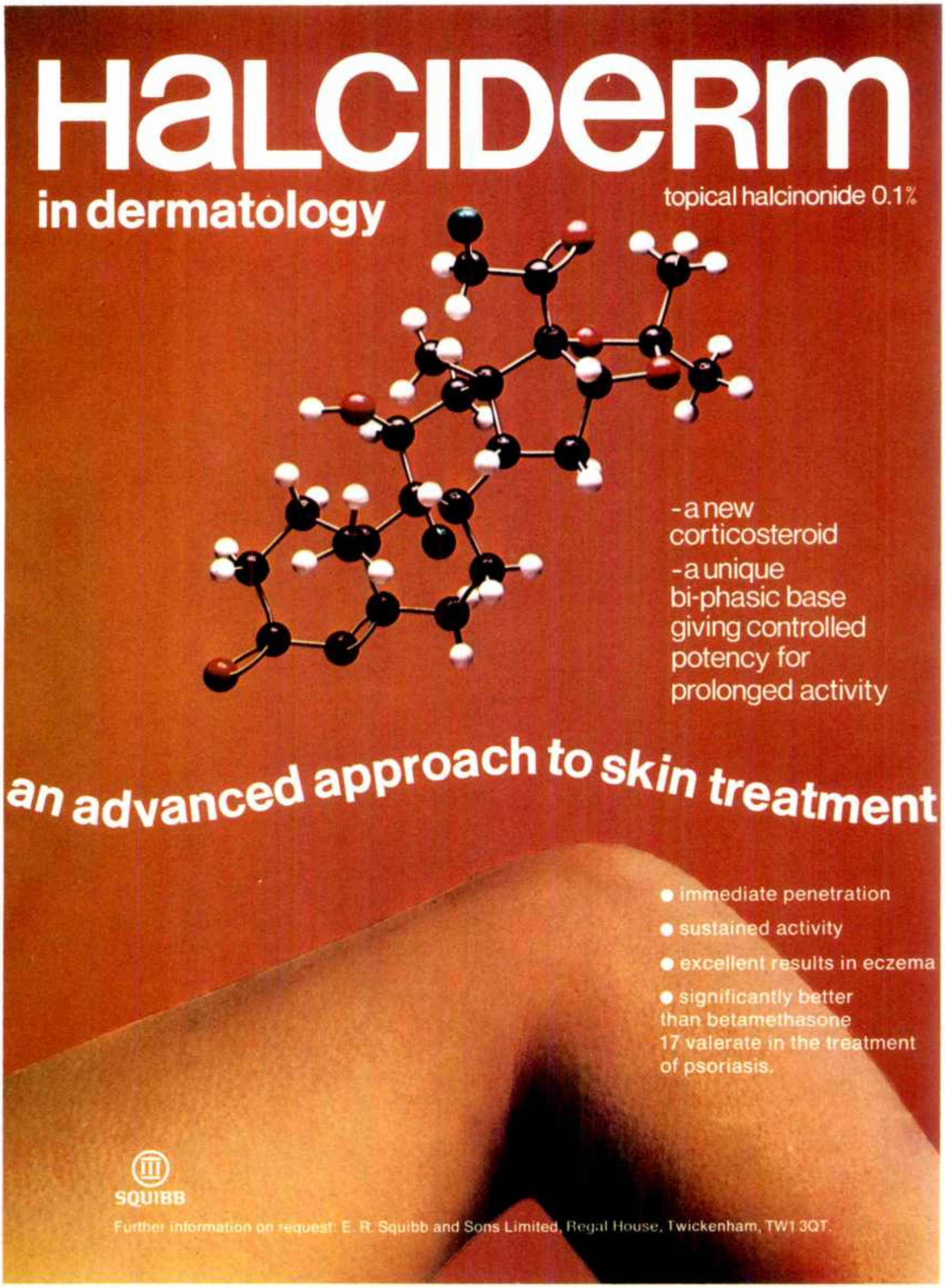

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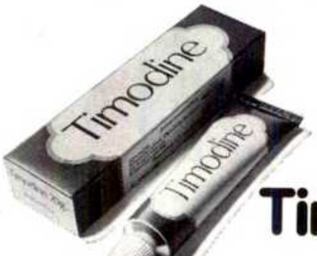

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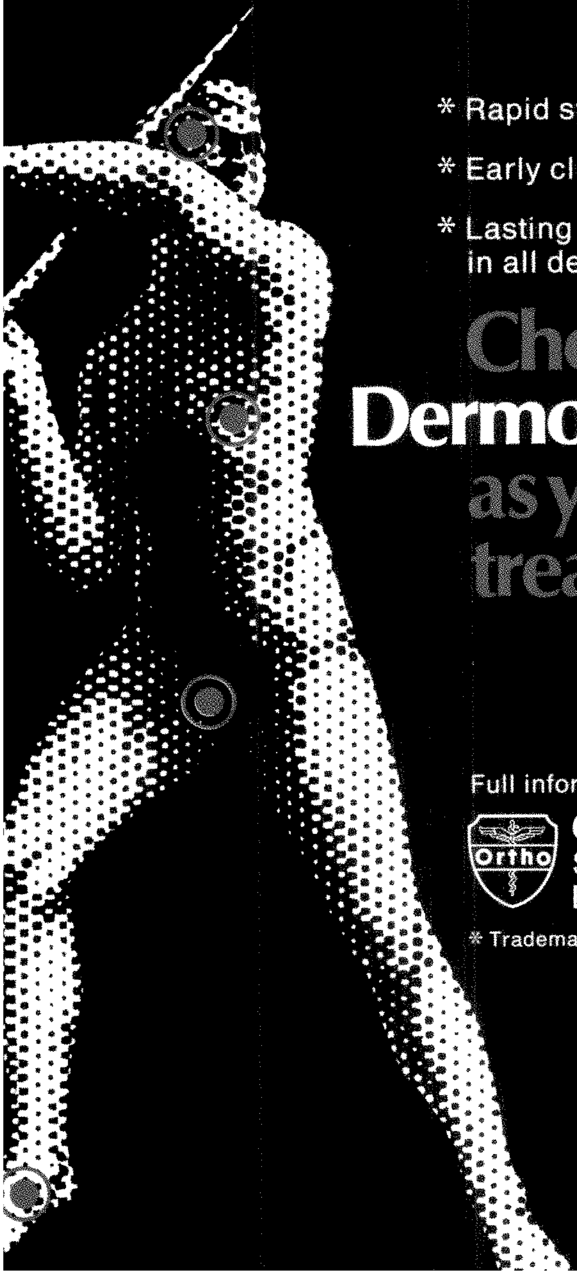

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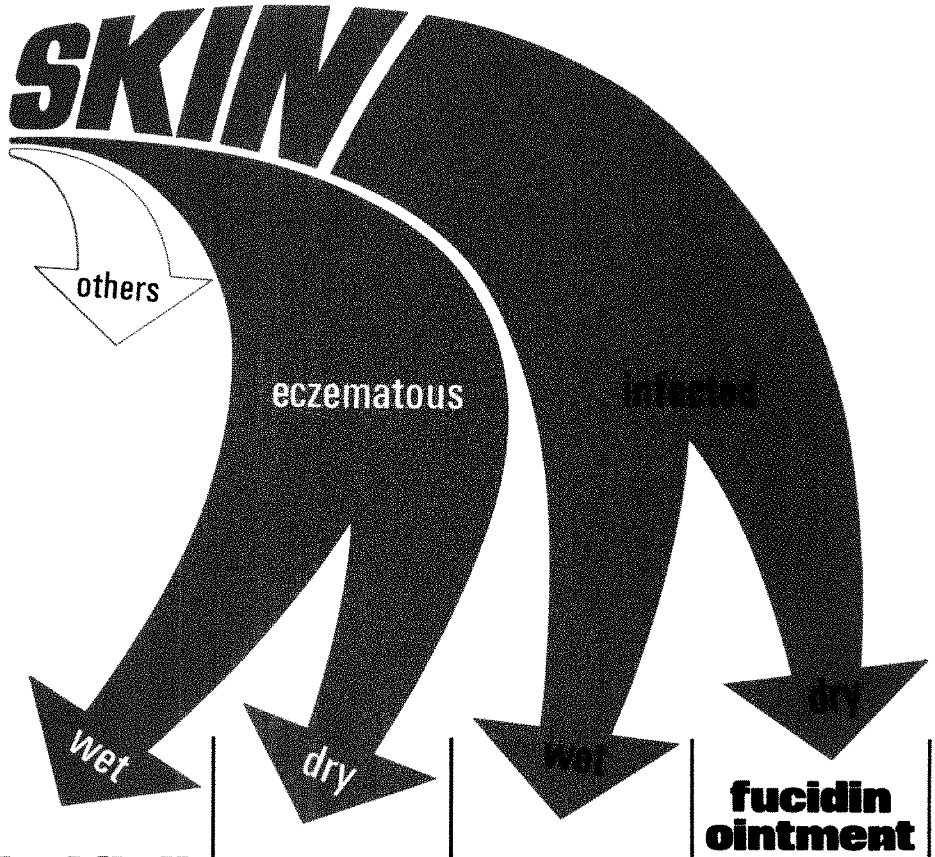

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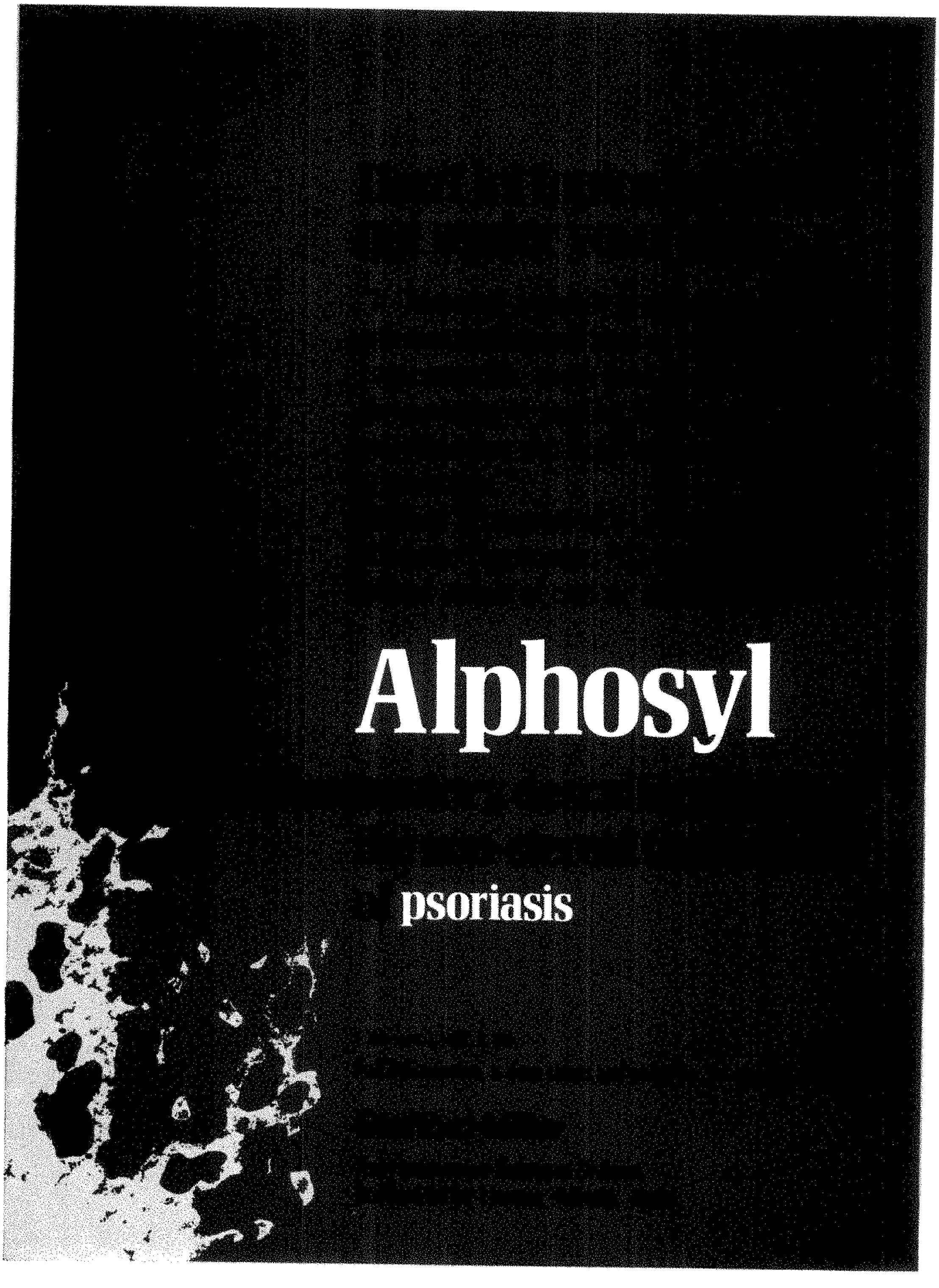
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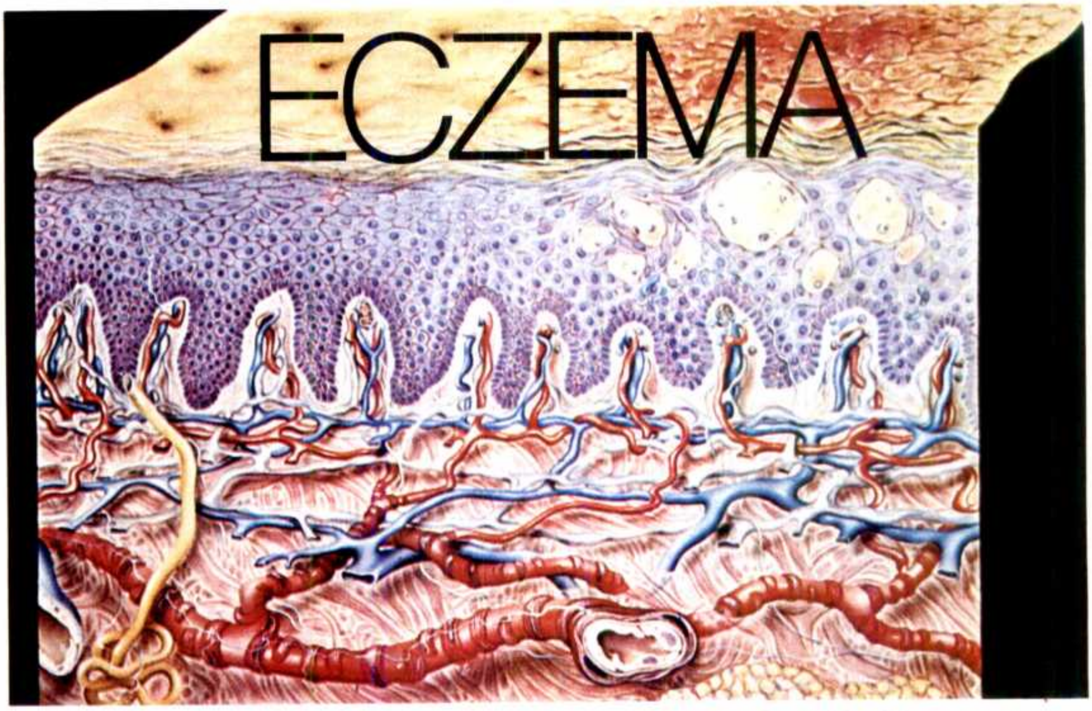

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British Journal of Dermatology (1975) **92**, 603.

Clinical and Laboratory Investigations

Phospholipase A and Lysophospholipase activity of the epidermis

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SUMMARY

Phospholipase A and lysophospholipase activities have been demonstrated in cow snout epidermis. The phospholipase A activity was dependent on Ca^{2+} ions and the pH for optimum activity was between 6·1 and 7·4.

Phospholipases (phosphatide acylhydrolases) catalyse the hydrolysis of diacylphosphoglycerides (phosphatides) to monoacylphosphoglycerides (lysophosphatides) and free fatty acid. The lysophosphatides may in turn be further hydrolysed by lysophospholipases (monoacylphosphoglyceride acylhydrolases) which remove the remaining fatty acid. Phospholipase A_1 and phospholipase A_2 are phosphatide acylhydrolases which hydrolyse specifically the fatty acyl ester linkages at the C_1 and C_2 hydroxyls of the glycerol moiety of the phosphatide respectively whereas phospholipase A is a phosphatide acylhydrolase of undefined positional specificity.

The phospholipases are widely distributed in nature and although they are found in highest concentration in snake venoms, extracts of higher plants and bacterial toxins, it is now evident that they are also widely distributed in animal tissues and bacteria. In addition to their extracellular role as constituents of venoms and digestive enzymes, they probably play an important part in the general intracellular metabolism of phospholipids in living organisms (McMurray & Magee, 1972).

The role of phospholipases in the epidermis has yet to be evaluated. The basal cell takes approximately 40 days to reach the stratum corneum (Halprin, 1972) and during this time it is transformed from a viable cell to a dead cell in which only the outer cell membrane and the keratin found within the cell remain. This transformation appears to be most rapid in the granular layer where degeneration of the nucleus and membranes of internal organelles of the cell takes place. The phospholipids are essential constituents of these membranes and catabolism of the phospholipids is obviously an important part of the process of keratinization as we have demonstrated a decrease in the phospholipid content of skin towards the skin surface (Long, 1970.)

This paper reports some further investigations on the phospholipase A activity of the epidermis which was demonstrated in earlier work (Long & Yardley, 1972).

MATERIALS AND METHODS

Analytical Methods

Protein was determined by the method of Lowry *et al.* (1951). Phospholipid phosphorus was determined by a modification of the method of Ernester, Zetterström & Lindberg (1950) which was described in a previous paper (Long & Yardley, 1970).

Scintillation counting of radioactive samples

The scintillation fluid was a solution of 10 g of 2-(4-tert-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole (butyl-PBD, BDH, England) in analytical grade toluene (1 litre). The fluid (10 ml) was added to each sample and the radioactivities were determined either by an IDL scintillation counter or by a Packard Tri-Carb liquid scintillation spectrometer (Model 527).

Solubilization of ^{14}C-phosphatidyl choline for incubation

The method of preparation and characterization of the ^{14}C-labelled phospholipid has already been described (Long & Yardley, 1972).

For each incubation an aliquot of the stock solution of ^{14}C-phosphatidyl choline (109 nmole; 3·4 μgP; $1{\cdot}38 \times 10^5$ counts/100 s) was evaporated to dryness with nitrogen, dissolved in diethyl ether (0·5 ml) and the appropriate sterile solution of buffer (1·0 ml) containing benzylpenicillin (100 units/ml) and streptomycin sulphate (50 units/ml) was added. The two phases were mixed continuously on a rotamixer and the ether phase slowly removed with a stream of nitrogen. In the experiments where the substrate was sonicated, the resultant clear solution was cooled in an ice-water bath and sonicated using a Dawe Soniprobe Type 1130 (Dawe Instrument Ltd; setting 2, 2 A) for a few minutes. The solution was centrifuged to remove any traces of metal from the probe.

Preparation and incubation of homogenates of epidermis from the cow snout

Cow snouts were obtained from the slaughter house, washed thoroughly with water and the epidermis was removed by taking two consecutive slices (each of 0·5 mm thickness) from the same area with a Castroviejo keratotome (John Weiss & Son, Wigmore Street, London, W.1). Pieces of full thickness epidermis (200–500 mg) were ground with the appropriate sterile solution of buffer (2 ml) containing benzyl penicillin (100 units/ml) and streptomycin sulphate (50 units/ml) in glass tissue grinders which were cooled in an ice-water bath. The epidermal homogenates were added to solutions of radioactive phosphatidyl choline in the same buffer, gassed with nitrogen, and incubated at 37°C for approximately 4 h on a shaker.

The buffer used routinely was 0·1 M borate pH 7 containing 2·5 mM calcium acetate. In the experiments with ethylenediamine tetra-acetic acid (EDTA) and p-chloromercuribenzoate (PCMB) added to the homogenates, the buffer used was 0·1 M glycylglycine pH 7·3.

Preparation of a cell-free fraction

Epidermis was obtained from the cow snout with a keratotome ($2 \times 0{\cdot}5$ mm slices taken consecutively from the same area), cut into small pieces with scissors and placed in the container of a Biox X-Press. The appropriate sterile solution of buffer or 0·9% sodium chloride solution was added and allowed to freeze at −20°C. The epidermis was homogenized by several passages through the orifice of the press at −20°C. The frozen homogenate was allowed to thaw and centrifuged at 2000 *g*. The supernatant was removed and the volume adjusted so that 1 ml was obtained from 200 to 300 mg of the epidermis. This solution was assayed for protein and aliquots of the solution were used as a crude enzyme preparation for incubation with ^{14}C-phosphatidyl choline.

Extraction of lipids from incubation mixtures

The lipids were extracted from the aqueous solutions of the assay system by shaking vigorously with four volumes of chloroform–methanol (2:1 v/v). After centrifugation, the upper aqueous layers were discarded and methanol added until the skin debris could be removed by centrifugation. The supernatants were removed and taken to dryness three times with additions of chloroform–methanol (2:1 v/v) to ensure dissociation of the proteo-lipids. The residues were redissolved in chloroform-methanol (2:1 v/v) and the extracts washed by the method of Folch, Lees & Sloane-Stanley (1957). The washed extracts were dried over anhydrous sodium sulphate.

Separation of the lipids

The total lipid extracts together with appropriate reference compounds were applied under nitrogen to a thin layer (0·25 mm) plate (20 × 20 cm) of silica gel G (Merck A.G., Darmstadt, Germany) which had been activated at 110°C for 30 min. The plates were developed either with light petroleum (boiling point range 30–40°C)–diethyl ether–glacial acetic acid (80:20:1 by vol.) or with chloroform–methanol–water (65:25:4 by vol.). After drying and exposing to X-ray film for 2–4 days, the plates were sprayed with an aqueous solution of rhodamine 6 g (0·01%) and the reference compounds, phosphatidyl choline, lyso-phosphatidyl choline and oleic acid were located with an ultraviolet lamp. The areas of silica gel containing the radioactive spots were scraped into scintillation vials and the radioactivity determined.

RESULTS

Composition of ^{14}C-phosphatidyl choline

The chemical and radiochemical compositions of the ^{14}C-phosphatidyl choline prepared from *Chlorella vulgaris* and purified on a column of DEAE-cellulose are shown in Table 1. The phospholipid which was highly unsaturated, contained a large proportion of oleic and linoleic acids, but the major radioactive compound was (1-^{14}C)-oleoyl phosphatidyl choline since about 96% of the radioactivity was present in oleic acid. The radioactivity was located only in the fatty acids of the phosphatidyl choline

TABLE 1. Composition of ^{14}C-phosphatidyl choline

Fatty acid	Fatty acid composition (% of total)	Radioactivity* (% of total)
14:0	0·8	0·2
16:0	12·6	0·4
16:1	3·4	0·0
16:2	3·1	0·1
18:0	4·1	1·4
18:1	36·3	95·6
18:2	33·9	1·4
18:3	1·1	0·1
Others	4·7	0·8

*Determined by radio-gas chromatography (Yardley & Long, 1972).

and 35% of the activity was present in the fatty acids esterified to the C1 hydroxyl and 65% in those esterified to the C2 hydroxyl.

Phospholipase activity in epidermal homogenates

Total epidermal homogenates were incubated with ^{14}C-phosphatidyl choline, and the lipids were extracted and separated by thin layer chromatography. Phospholipase activity was determined by measuring the radioactivity in the breakdown products and in the undegraded phosphatidyl choline. Sonication of the substrate had no effect on the release of free fatty acids from the ^{14}C-phosphatidyl choline in a 4½ h incubation. 63% of the radioactivity was recovered in the free fatty acids released from sonicated substrate and 60% in the free fatty acids from ^{14}C-phosphatidyl choline which had not been sonicated. Only 1% of the recovered radioactivity was present in lysophosphatidyl choline. Boiling the homogenates prior to incubation prevented the release of free fatty acids from the substrate. There was no significant release of free fatty acids from glyceryl-(1-^{14}C) trioleate when incubated under the same experimental conditions.

Storage of skin at −20°C overnight had no effect on the phospholipase activity and skin stored at −20°C for 3 weeks retained similar levels of activity. Addition of EDTA (final concentration $2{\cdot}5 \times 10^{-3}$ M) to the homogenates produced 75% inhibition of activity and the addition of PCMB (final concentration $2{\cdot}5 \times 10^{-4}$ M) produced 38% inhibition of activity.

Homogenates were clarified by centrifugation at 2000 *g* and phospholipase activity was detected in the cell-free fraction and in the insoluble residues. The degradation products produced by the cell-free fraction were identical to those in the total homogenates and so the cell free fraction was used in subsequent experiments to determine some of properties of the enzyme activity.

Phospholipase A activity of the cell-free fraction

Effect of Ca^{2+} on activity. The phospholipase activity was dependent on Ca^{2+} ions and the optimum Ca^{2+} concentration was 15 mM (Fig. 1).

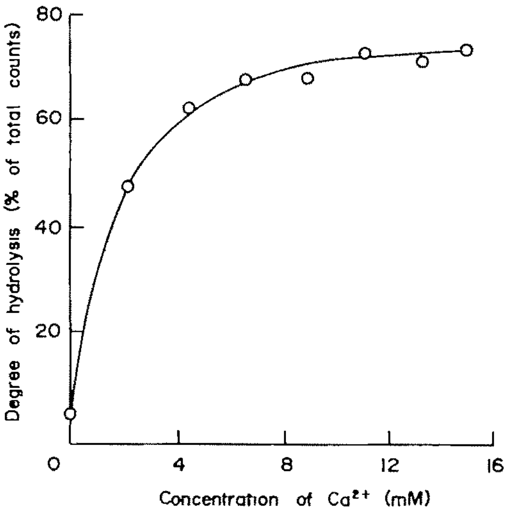

FIGURE 1. Effect of Ca^{2+} on the hydrolysis of ^{14}C-phosphatidyl choline (95 nmoles) in 0·1M borate buffer pH 7 (3ml). Time of incubation = 4 h at 37°C.

Effect of pH on activity

The pH for optimum activity was between 6·1 and 7·4 (Fig. 2).

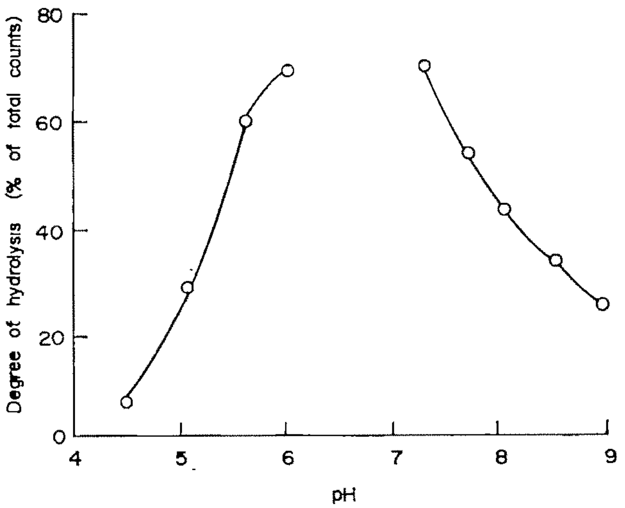

FIGURE 2. Effect of pH on the hydrolysis of ^{14}C-phosphatidyl choline (95 nmoles) in 0.1 M acetate or borate buffer (3ml) containing 15mM Ca^{2+}. Time of incubation, 2½ h at 37°C.

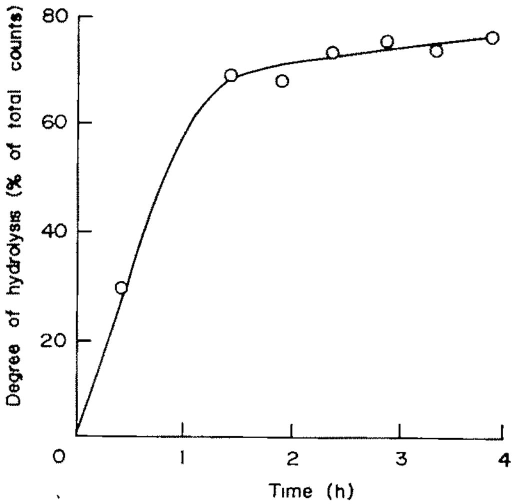

FIGURE 3. Time curve for the hydrolysis of ^{14}C-phosphatidyl choline (95 nmoles) in 0.1 M borate buffer pH 7 (3ml) containing 15 mM Ca^{2+} at 37° C.

Effect of time and protein concentration

The time curve (Fig. 3) shows that hydrolysis of the ^{14}C-phosphatidyl choline was virtually complete after 1½ h of incubation and linear with time during the initial hour of incubation. The rate of hydrolysis of the ^{14}C-phosphatidyl choline was 17 nmoles/mg protein/h. The protein concentration curve (Fig. 4) shows that the degree of hydrolysis was proportional to the protein concentration of the enzyme solution when a short incubation time (40 min) was used.

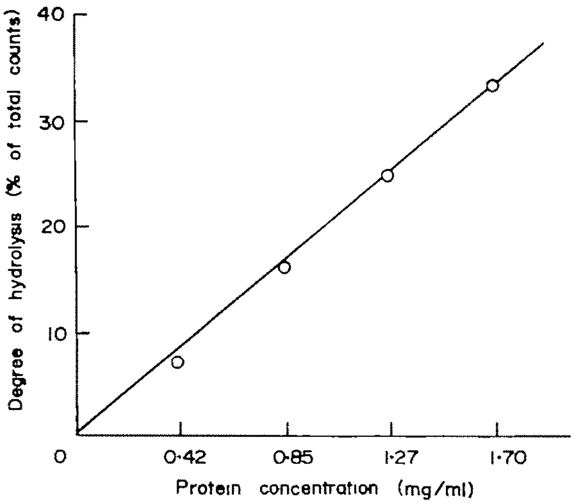

FIGURE 4. Protein concentration curve for the hydrolysis of ^{14}C-phosphatidyl choline (95 nmoles) in 0.1M borate buffer pH 7 (4.5ml) containing 15mM Ca^{2+}. Time of incubation, 40 min at 37°C.

DISCUSSION

When homogenates of epidermis were incubated with the ^{14}C-phosphatidyl choline, less than 1% of the radioactivity was recovered in lysophosphatidylcholine. This suggests that the substrate is hydrolysed initially by a rate-limiting phospholipase A and that the lysophosphatide that is formed is rapidly hydrolysed by a corresponding lysophospholipase. Studies on the respiration of skin slices in the absence of added glucose have shown that although skin cells degrade endogenous phospholipid, the phospholipid composition is unchanged and there is no accumulation of lysophosphatides (Long & Boyce, 1971). It is possible that the hydrolysis could be caused by a single enzyme which hydrolyses both acyl ester linkages simultaneously (termed a phospholipase B), but recent studies suggest that phospholipase B is an undissociated phospholipase A–lysophospholipase complex in which the latter enzyme predominates (McMurray & Magee, 1972). The activity cannot be attributed to lipase action as the hydrolysis of glyceryl-(1-^{14}C) trioleate was hardly measurable when incubated under the same experimental conditions.

Storage of skin at −20°C for several weeks did not affect the phospholipase activity of the homogenates. The phospholipase activity was inhibited by EDTA which suggested that a metal ion was required for activity. The residual activity that was present in the absence of added Ca^{2+} was inhibited to some extent by PCMB. However, it is possible that some of this residual activity could be due to contaminating lipase activity since liver lipase is inhibited by PCMB whereas liver phospholipase A_1 and A_2 activities are unaffected (Waite & van Deenen, 1967). The phospholipase of intestinal mucosa is, however, reversibly inactivated by PCMB and the addition of sulphydryl compounds increases the activity (Ottolenghi, 1964).

The phospholipase activity was dependent on Ca^{2+} ions. In the absence of added Ca^{2+} ions, there was some small residual activity, but this could well be due to Ca^{2+} ions present in the enzyme preparation. The Ca^{2+} ion dependence and the pH optimum near neutrality for the epidermal phospholipase A are similar to the properties of phospholipase A_2 preparations from a wide range of mammalian sources, such as arterial tissue (Eisenberg, Stein & Stein, 1968), heart muscle (Weglicki *et al.*, 1971) and rat liver (Waite & Sisson, 1971).

Phospholipase A seems to be present in every subcellular fraction of the cell, but the positional specificity and properties of the enzyme frequently correlate with its subcellular localization. Thus subcellular fractionation of rat liver homogenates showed that phospholipase A_1 activity was mainly located in the microsomes, phospholipase A_2 in the mitochondria and lysophospholipase in the soluble fraction (Waite & van Deenen, 1967). Subsequent work has shown that rat liver lysosomes contain phospholipases A_1 and A_2 and lysophospholipase (Mellors & Tappel, 1967). The lysosomal phospholipases have acidic pH optima (4–5) and are inhibited by Ca^{2+} ions (Franson, Waite & La Via, 1971), whereas the mitochondrial phospholipase A_2 is stimulated by Ca^{2+} ions and has an alkaline pH optima of 7–8 (Waite & Sisson, 1971). This would suggest that the epidermal phospholipase A does not originate from the lysosomes. The lysosomal phospholipases are invariably either unaffected by or inhibited by Ca^{2+} ions and have optimal activity at pH 4–5.

The rather broad pH curve with an optimum between 6·1 and 7·4 and the marked shoulder at alkaline pH values would suggest that there is more than one acylhydrolase present in the enzyme preparation. The pH optimum differs from the value of 8·3 which was found in previous work (Long & Yardley, 1972) when extracts prepared from acetone powders of the epidermis were used. However, the phospholipase activities of the extracts from acetone powders were determined in the presence of sodium deoxycholate which is known to alter the pH optimum towards alkaline values (de Haas *et al.*, 1971).

It remains to be established whether the phospholipase A activity described in this paper plays an important role in the process of keratinization. In order to investigate this problem satisfactorily it will be necessary to prepare different populations of cells by a method of sequential trypsinization similar to that used by Vaughan & Bernstein (1971). The phospholipase activities of populations of basal cells, spinous cells, granular cells and squamous cells could then be investigated. Likewise, the subcellular localization of the various phospholipases of the epidermal cell can only be investigated when adequate preparations of subcellular organelles can be obtained.

ACKNOWLEDGMENT

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Humoral and cellular immunity in atopic eczema

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SUMMARY

Parameters of humoral and cellular immunity were measured in thirty-five patients with atopic eczema. The mean serum IgE level was raised but levels of the other major immunoglobulin classes were normal. Ten per cent of patients failed to respond to tetanus immunization. All patients responded to *S. typhi* H antigen. Fourteen per cent of patients failed to mount delayed hypersensitivity reactions to a battery of three intradermal antigens. The phytohaemagglutinin-stimulated uptake of ^{3}H thymidine by lymphocytes was normal in the presence of autologous or of fetal calf serum, as was the spontaneous lymphocyte uptake. T and B lymphocyte numbers in the peripheral blood were normal. These results are similar to those found in asthmatic patients and support the hypothesis that, in some patients, atopic eczema is associated with an immunodeficiency state.

Immunoglobulin E antibodies are associated with the atopic disorders, asthma, hay fever and atopic eczema. There is variability in the allergens to which an atopic individual will develop hypersensitivity and variation in the organs which are affected. The fundamental reason or reasons for clinical manifestations in a proportion of the population is still not clear. Several hypotheses, not necessarily mutually exclusive, have been put forward to explain the development of the atopic state. The familial clustering of atopic disease suggests that an atopic tendency may be inherited (Sherman, 1965). Increased sensitivity to pharmacological intermediates released from mast cells or basophils as a result of the interaction of antigen with surface IgE may be an underlying fault in atopy (Townley, Dennis & Itkin, 1965). It has been suggested that in asthma there is a functional imbalance of the autonomic nervous system with partial blockade of the beta adrenergic system (Szentivanyi, 1968). There may be a defect in the mucosal barrier in atopic subjects leading to more efficient contact between antigen and immunologically competent cells (Leskowitz, Salvaggio & Schwartz, 1972).

In 1937, Rostenberg & Sulzberger showed that patients with atopic dermatitis were much less likely to have delayed hypersensitivity reactions on patch testing with a wide range of antigens than patients with contact dermatitis. Kaufman & Hobbs (1970) have suggested that this may be the first record of an association between immune deficiency and atopy. Kuhns & Pappenheimer (1952) described subjects who produced reaginic but not precipitating antibodies to diphtheria toxoid. A high prevalence of atopy has been noted in immunoglobulin deficiency syndromes (Hobbs, 1969). Conversely immunoglobulin deficiencies, especially of IgA, were found in more than 7% of atopic subjects (Kaufman & Hobbs, 1970). They suggested that 'atopy develops in individuals whose immunological vocabulary is backward'. Taylor *et al.* (1973) have shown that transient IgA deficiency in infancy may be associated with the subsequent development of atopy in the children of reaginic parents.

We have reported that some patients with asthma have deficiencies of humoral or cellular immunity as shown by impaired antibody response to tetanus toxoid, impaired delayed hypersensitivity reactions to antigen and altered lymphocyte response to phytohaemagglutinin when cultured in fetal calf serum (Grove *et al.*, 1975). This study was undertaken to determine whether abnormalities of humoral and cellular immunity could be demonstrated in atopic eczema.

Patients and controls

Twelve male and twenty-three female patients were studied. Ages ranged from 5 to 64 years, with 1 patient in the first decade and 6, 18, 6, 1, 2 and 1 patients in each succeeding decade.

All patients had been referred for dermatological opinion and had been diagnosed as suffering from atopic dermatitis on the basis of the clinical history and morphology and distribution of the lesions. Patients had either the typical flexural eczematous lesions with lichenification, or atypical dermatitis with a definite past history of classical infantile eczema plus a past or family history of asthma or hay fever.

Control values were established on normal subjects with a similar age and sex distribution to the patients studied. As an additional control, lymphocyte tritiated (^{3}H) thymidine uptakes were measured in patients with psoriasis, some of whom were receiving topical corticosteroid therapy.

METHODS

Immunoglobulin levels

Serum levels of immunoglobulins (Ig) G, A and M were measured with Behringwerke immunodiffusion plates. Standard solutions were obtained from Behringwerke. Serum levels of IgD and IgE were measured by the single radial diffusion method described by Rowe (1969). IgD standard was obtained from Behringwerke; IgE standard was from a batch of pooled human sera, 69/204, supplied by WHO.

Antibody responses

Patients were immunized with tetanus toxoid (Commonwealth Serum Laboratories, C.S.L.) 0·5 ml s.c. and typhoid vaccine (C.S.L.) 0·1 ml s.c.; blood was collected before, and 2 weeks after immunization. Haemagglutinating antibodies to tetanus antigen, and precipitating antibodies to *S. typhi* H antigen, were measured as previously described (Forbes, 1971).

Most South Australians have previously been exposed to tetanus toxoid, but have not usually been immunized with typhoid vaccine. Mercaptoethanol treatment of sera from normal subjects, patients with chronic infections, patients with dystrophia myotonica, and asthmatics confirmed that response to tetanus toxoid was usually of the IgG type, while *S. typhi* H antigen produced an IgM response (Forbes, 1971).

Delayed hypersensitivity reactions

Delayed hypersensitivity (DHS) reactions to intradermal injections of 0·1 ml of *Candida albicans* (1%) (Bencard), Mumps Skin Test Antigen (Eli Lilly), and Streptokinase–Streptodornase ('Varidase', Lederle) containing 10 units of streptokinase and 2·5 units of streptodornase were measured at 48 hours. An area of induration of 5 mm or more was considered positive. Patients were considered negative if they failed to react to at least one antigen. Tests were not performed on areas of dermatitis, nor on areas receiving topical corticosteroids.

Lymphocyte tritiated thymidine uptake

Phytohaemagglutinin (PHA)-induced uptake of ^{3}H thymidine by lymphocytes was measured in whole blood culture. Dose response curves to PHA were established. The ^{3}H thymidine uptake at varying periods of incubation with the PHA dose producing maximum responsiveness was measured. On the basis of these results, the most appropriate PHA dosage and period of incubation were used as described below.

Cultures contained 0·2 ml heparinized whole blood, 3·4 ml HEPES-buffered medium 199 (C.S.L.), 0·02 ml PHA (Wellcome, Reagent Grade) and 0·4 ml of fetal calf serum (C.S.L.) or autologous serum. Triplicate cultures were incubated for 4 days at 37°C. At 92 hours, 2·5 μCi ^{3}H thymidine (specific activity: 500 mCi/mmol) were added and incubated for another 4 h. Cultures were then kept at 4°C for 30 min; centrifuged at 250 *g* for 10 min, washed with 3% acetic acid, 0·9% saline, and 5% trichloracetic acid in 0·45% saline, in succession; centrifuged at 1,000 *g* for 20 min with 5% trichloracetic acid and twice with methanol. After drying, the residue was dissolved in 0·5 ml Soluene (Packard) for liquid scintillation counting.

The spontaneous uptake of ^{3}H thymidine by cultures, i.e. in the absence of PHA, was measured by incubating 0·2 ml of whole blood in 3·8 ml of medium with 2·5 μCi of ^{3}H thymidine at 37°C for 4 h then treated as above. Results were expressed as disintegrations per culture per minute.

B and T lymphocytes

B cells were measured by the method of Froland & Natvig (1971) and T cells by the method of Wybran Chantler & Fudenberg (1973).

Ten ml of heparinized blood was diluted with 20 ml of Dulbecco buffer pH 7·4 (C.S.L.) and layered on 10 ml of Ficoll/Hypaque mixture, 12 parts of 9% Ficoll (Pharmacia) in distilled water and 5 parts of 33% Hypaque (Winthrop), in a glass centrifuge tube then spun for 40 min at 400 *g*. The lymphocyte layer, containing 90–95% lymphocytes, was removed, spun down and washed twice with Dulbecco buffer. Cells were resuspended in buffer and divided into five aliquots.

One drop of antiserum to IgG, IgA and IgM (Hyland) and polyvalent antiserum (Roboz) was added to each tube with two drops of heat-inactivated fetal calf serum (C.S.L.). The tubes were incubated for 1 h at 4°C, washed three times with Dulbecco buffer, resuspended in fetal calf serum, then the percentage of fluorescing lymphocytes counted under U.V. light with a Zeiss microscope. The specificity of the serum was confirmed by blocking experiments with unlabelled antiserum.

Sheep red cells (C.S.L.) were washed three times in saline. A suspension of 2,000,000 lymphocytes was added to 1 ml of 0·5% sheep red cells and 3 drops of heat-inactivated fetal calf serum in a centrifuge tube. The cells were then spun at 150 *g* for 5 min and kept at 4°C for 1 h. The cells were resuspended gently and 1 drop of toluidine blue added. The percentage of rosette-forming cells was counted on a Neubauer counting chamber.

RESULTS

Immunoglobulin levels

IgE levels were raised in patients with atopic eczema, while levels of IgG, IgA, IgM and IgD were normal (Table 1). Thirty-seven per cent of patients had IgE levels above the normal range.

Antibody responses

Antibody titres were measured before and after immunization in thirty-one patients. Haemagglutinating antibodies to tetanus toxoid were not detectable in three (10%) of the patients. Each patient was age and sex matched with two controls, all of whom responded to immunization. This difference was significant ($P < 0·04$, Fisher's exact test).

TABLE 1. Serum immunoglobulin levels

	Number	Mean	s.d.	Probability*
Patients IgG	35	1460	320	N.S.
Controls	35	1560	325	
Patients IgA	35	166	60	N.S.
Controls	35	195	77	
Patients IgM†	35	172	73	N.S.
Controls	35	170	66	
Patients IgD†	35	12	99	N.S.
Controls	35	13	80	
Patients IgE†	35	348	1780	<0·001
Controls	35	100	107	

* Probability by '*t*' test. † Geometric mean. IgG, IgA and IgM: mg/100 ml. IgD, IgE: units/ml. N.S., not significant.

Precipitating antibodies to *S. typhi* H antigen were found in all patients and controls.

Delayed hypersensitivity reactions

Fourteen per cent of patients failed to react to any of the three intradermal antigens, compared with no failure in the controls (Table 2). Atopic eczema patients reacted to 55% while controls reacted to 73% of all the tests performed.

TABLE 2. Delayed hypersensitivity reactions

	Reactors	Non-Reactors	Probability
(a) Patients who reacted to at least one antigen			
Patients	30	5	<0·03
Controls	35	0	
(b) Total number of reactions to the three antigens			
Patients	58	47	<0·001
Controls	77	28	

Probability by Fisher's exact test.

Lymphocyte tritiated thymidine uptake

The uptake of ^{3}H thymidine by lymphocytes is shown in Table 3. There was no significant difference in uptake, either spontaneously or stimulated with PHA when cultured in either autologous or fetal calf serum. There were no significant differences in any of the parameters between the twenty-two patients using betamethasone cream and the twelve not using a steroid preparation. Similarly there were no differences in PHA-stimulated uptake between twenty-six psoriatic patients using, and thirteen psoriatic patients not using, steroid preparations.

There were no significant differences in the PHA-stimulated uptake of DHS non-reactors compared with DHS reactors.

TABLE 3. Lymphocyte ^{3}H thymidine uptake*

		Number	Mean	s.d.	Probability
PHA ^{3}H uptake —autol.	Patients	34	51,000	26,000	N.S.
	Controls	34	55,800	27,000	
PHA ^{3}H uptake —F.C.S.	Patients	30	58,000	22,000	N.S.
	Controls	30	51,500	20,000	
Spontaneous —^{3}H uptake	Patients	35	420†	470	N.S.
	Controls	35	376†	155	

* Disintegrations per culture per min. † Geometric mean. Probability by '*t*' test. N.S., not significant.

T and B lymphocytes

There were no significant differences in either the absolute lymphocyte count, T cell numbers, or total IgG, IgA and IgM B cell numbers (Table 4).

TABLE 4. T and B lymphocytes: eosinophils*

		Number	Mean	s.d.	Probability
Lymphocytes	Patients	34	1950	750	N.S.
	Controls	34	1840	410	
T cells	Patients	34	920	490	N.S.
	Controls	34	910	350	
B cells	Patients	34	280	175	N.S.
	Controls	34	220	100	
IgG	Patients	34	170	100	N.S.
	Controls	34	140	60	
IgA	Patients	34	74	56	N.S.
	Controls	34	78	48	
IgM	Patients	34	150	100	N.S.
	Controls	34	150	80	
Eosinophils	Patients	34	160†	1500	<0 001
	Controls	34	42†	500	

* Cells/μl. † Geometric mean. Probability by '*t*' test. N.S., not significant.

Eosinophils

There was a significantly higher eosinophil count in patients than controls (Table 4).

DISCUSSION

These results provide evidence of impairment of antibody production or cellular immune processes in some patients with atopic eczema. They are consistent with a well-known clinical observation which suggested the possibility of immunodeficiency in atopic eczema. Kaposi's varicelliform eruption usually results from infection with herpes simplex or vaccinia viruses in patients with atopic eczema (Rook, Wilkinson & Ebling, 1972) suggesting that defence mechanisms are impaired. Moreover, many patients with eczema vaccinatum respond to hyperimmune antivaccinial gamma globulin (Sharp & Fletcher, 1973).

The mean IgE level was elevated as has been shown in other studies (Juhlin, Johansson & Bennich, 1969; Stone, Muller & Gleich, 1973). This elevation was greater than that reported by us (Grove *et al.*, 1975) in asthmatic patients, as has been observed by others (Johansson *et al.*, 1970; Wood & Oliver, 1972). Serum IgG levels were normal, in contrast to the observations of Varelzidis *et al.* (1966) who found raised levels.

Ten per cent of patients failed to respond to immunization with tetanus toxoid. This represents not merely failure to increase titre but failure to produce any detectable haemagglutinating antibodies 2 weeks after immunization. The response to *S. typhi* H antigen was normal. Response to tetanus toxoid may be a relatively sensitive indicator of humoral immunological competence or reflect the greater thymus-dependence of tetanus antigen (Hess, 1968) compared with Salmonella flagellar antigen (Davies *et al.*, 1970).

Fourteen per cent of patients failed to make DHS reactions to intradermal antigen. Moreover, there was a significant reduction in the total number of reactions to antigens compared with normal subjects. These findings support those of Rajka (1970) who showed that delayed reactions to bacterial and viral extracts were impaired in atopic eczema. It may be expected that patients with a cellular immune defect would have less contact dermatitis. Since the original observations of Rostenberg & Sulzberger, there has been considerable controversy over the prevalence of contact dermatitis in atopic eczema. A Scandinavian committee tested 1027 patients with a routine series of patch tests and found that 13% of atopics and 31% of non-atopics had positive reactions (Magnusson *et al.*, 1969). Calnan (1956) and Caron (1964) found little evidence of atopy in nickel sensitivity. Other authors have concluded that atopic individuals were no more likely to develop contact dermatitis than normal persons (Wilson, 1955; Jillson, Curwen & Alexander, 1959; Fregert & Moller, 1963; Cronin *et al.*, 1970). In contrast, some authors thought that there was a positive relationship between atopy and contact dermatitis (Epstein & Mohajerin, 1964; Epstein, 1965; Forsbeck, Skog & Ytterborn, 1966). These interpretations, however, may reflect the greater exposure of patients with atopic eczema to topical medicaments, and any damaged skin is more likely to develop a contact dermatitis (Rook *et al.*, 1972).

No abnormalities of lymphocyte ^{3}H thymidine uptake were seen in patients with atopic eczema. Similarly, Fjelde & Kopecka (1967) did not demonstrate a significant reduction in PHA-stimulated lymphocyte transformation assessed morphologically. Topical corticosteroid therapy appeared to have no effect on lymphocyte activity either in patients with atopic eczema or in those with psoriasis. There were no significant abnormalities in either T or B lymphocyte numbers.

The observations of Lobitz, Honeyman & Winkler (1972) support the concept of immunodeficiency in atopic dermatitis. They reported two adult patients with atopic dermatitis who had depressed cell-mediated immunity as evidenced by delayed hypersensitivity reactions on skin testing, inability to be sensitized with dinitrochlorobenzene, reduced lymphocyte response to PHA, and lymphadenopathy. Moreover, patients with the Wiskott–Aldrich syndrome frequently have an atopic type of eczema in association with impaired delayed hypersensitivity reactions (Cooper *et al.*, 1964), and have been shown to have raised serum IgE levels (Berglund *et al.*, 1968).

The data presented parallel the observations in asthmatics (Grove *et al.*, 1975), although there were some differences. IgG levels were elevated and PHA-stimulated uptake of lymphocytes cultured in fetal calf serum was depressed in asthmatics. It is of interest that this depression was less in patients with a past history of eczema. More asthmatics failed to respond to tetanus immunization and DHS skin reactivity was less impaired than in patients with atopic eczema. These variations in the frequencies of different abnormalities may be chance occurrences or related to the different clinical manifestations of atopy.

These data support the hypothesis that in some patients with atopy there are deficiencies in the immunological defence mechanisms leading to increased antigenic stimulation of the reaginic system.

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On the lack of response to catecholamine stimulation by the adenyl cyclase system in psoriatic lesions*

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SUMMARY

When epidermis from the uninvolved skin of psoriatic patients was incubated for 5 min in Hank's medium containing adrenaline and theophylline, the cyclic AMP level consistently increased 20–30 times over the level observed when adrenaline was not added to the medium. On the other hand, when epidermis from the involved skin of psoriatic patients was incubated under the same experimental conditions, the cyclic AMP level increased only 2–5 times. Even when theophylline, an inhibitor of specific cyclic AMP-phosphodiesterase, was omitted from the medium, a clearly demonstrable difference in sensitivity to adrenaline was evident in normal appearing and lesional psoriatic epidermis. These results indicate a faulty adenyl cyclase system in the involved epidermis of psoriatic lesions rather than a defective degradation process by the specific phosphodiesterase. Since the Km for adrenaline activation of adenyl cyclase was approximately the same in both the uninvolved and the involved epidermis and since the cyclic AMP increase by adrenaline was abolished by the addition of propranolol, the basic nature of the β-receptor (specifically the binding affinity to adrenaline) in the involved epidermis does not appear to be defective. On the other hand, the finding that the V_{max} for adrenaline activation is 10–20 times higher in the uninvolved than in the involved epidermis suggests that the poor response in the involved epidermis may be due to fewer available binding sites for adrenaline in the psoriatic lesion.

Whether or not epidermis from psoriatic lesions has a defective adenyl cyclase system has been a controversial issue. Voorhees *et al.* (1973) reported that adenyl cyclase in psoriatic lesions did not appear to be defective in its response to a β-agonist, isoproterenol. But Wright *et al.* (1973) demonstrated a sluggish response of adenyl cyclase in psoriatic epidermis towards another β-agonist, adrenaline. This study was undertaken to determine whether the hormonal regulation is defective in psoriasis.

MATERIALS AND METHODS

Since the major aim of this study was to compare the hormonal (catecholamine) sensitivity of the

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uninvolved and involved epidermis from psoriatic patients, we avoided the use of homogenized epidermal samples or of membrane preparations, since the disruption of cells reportedly causes the loss of hormone sensitivity (Robison, Butcher & Sutherland, 1971). Epidermal samples were obtained with a keratotome, set at 0·2 mm depth, from about a dozen adult male patients with well-developed psoriatic lesions. No systemic or local anaesthesia was given, and no antiseptic was applied topically before the biopsy. Pieces of epidermis measuring about 5 mm × 5 mm were pre-incubated in Hank's balanced salt solution at 37°C for 15–20 min to stabilize the cyclic AMP and ATP levels and then incubated in Hank's medium with and without adrenaline and with and without theophylline. Changes in cyclic AMP levels in the epidermis were measured by a modification of Gilman's protein binding method (Yoshikawa *et al.*, 1975). Generally two pieces of the epidermis per tube were used for each assay.

RESULTS

The time course of adrenaline stimulation of the cyclic AMP increase in the uninvolved and involved

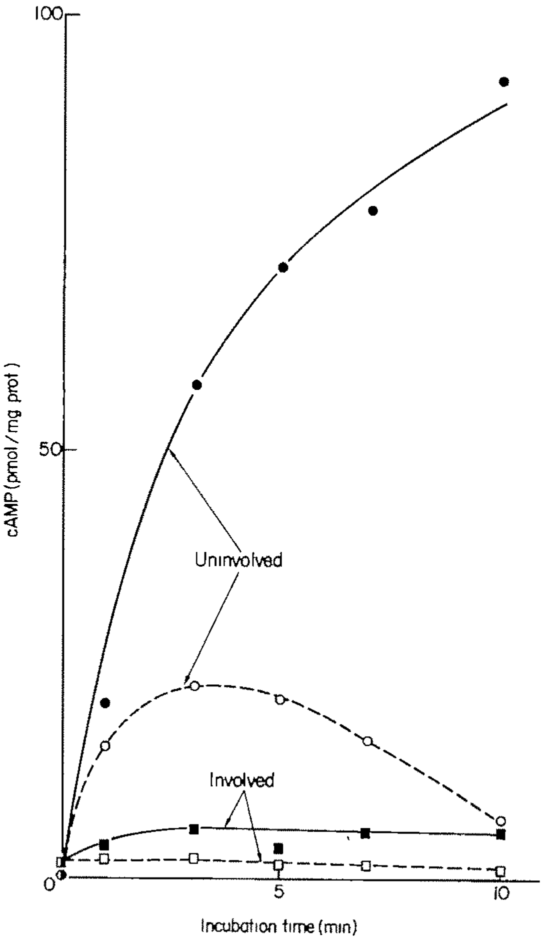

FIGURE 1. Time course of changes in the cyclic AMP levels in the uninvolved and involved epidermis. ●, ■: Adrenaline ($4{\cdot}5 \times 10^{-6}$M) and theophylline (2 mM) were added to Hanks' medium. ○, □: Adrenaline ($4{\cdot}5 \times 10^{-6}$M) alone.

epidermis from the same psoriatic patient is shown in Fig. 1. Without the addition of the specific phosphodiesterase inhibitor theophylline, the cyclic AMP level was clearly elevated in the uninvolved epidermis, reaching a peak during the first 3–5 min and then falling. When theophylline was added together with adrenaline, the cyclic AMP level in the uninvolved epidermis continued to increase for 10 min. With or without added theophylline the response to adrenaline by the involved epidermis was only slight as compared with the uninvolved.

The effect on the cyclic AMP increase of varying the adrenaline concentration is shown in two patients in Fig. 2. Despite some variation in the uninvolved epidermis, the difference in the response curve between the uninvolved and the involved epidermis is obvious. Apparent Kms for adrenaline in the uninvolved epidermis in both experiments are $3{\cdot}7\times10^{-7}$M. The Km for the involved epidermis could not be calculated accurately because of a minimum increase in cyclic AMP, but approximation with the Lineweaver–Burk plot yielded about 4×10^{-7}M for case A and $2{\cdot}5\times10^{-7}$M for case B. From the same double-reciprocal plots for cases A and B, we calculated the V_{max} values for the uninvolved and involved epidermis to be 180 and 17 pmol/mg protein respectively in case A and 80 and 3 pmol/mg protein respectively in case B.

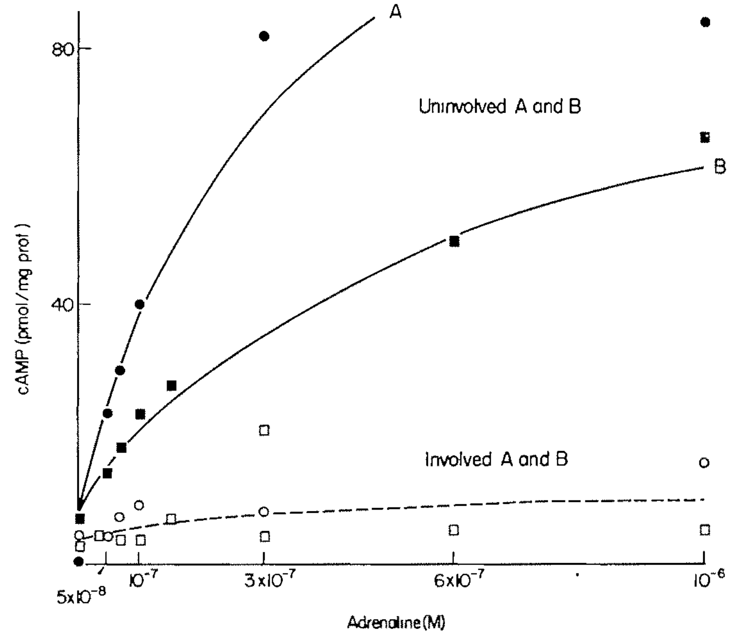

FIGURE 2. Effects of adrenaline concentration on the cyclic AMP levels in the uninvolved and involved epidermis. Results of two experiments (A and B) are shown. The incubation time was 5 min.

The responses to adrenaline in the uninvolved and involved epidermis from psoriatic patients are compared in Fig. 3. The addition of theophylline (2 mM) alone to the medium did not change the cyclic AMP level during the 5 min incubation, but the addition of theophylline plus adrenaline caused an average increase of 27·5- and 5-fold in the uninvolved and involved epidermis respectively, or a difference of 550%. When only adrenaline was added to the medium, a substantial increase in cyclic AMP was still observed. The average increase in the uninvolved epidermis was 8·5-fold, that in the involved epidermis 2-fold, or a difference of 425%.

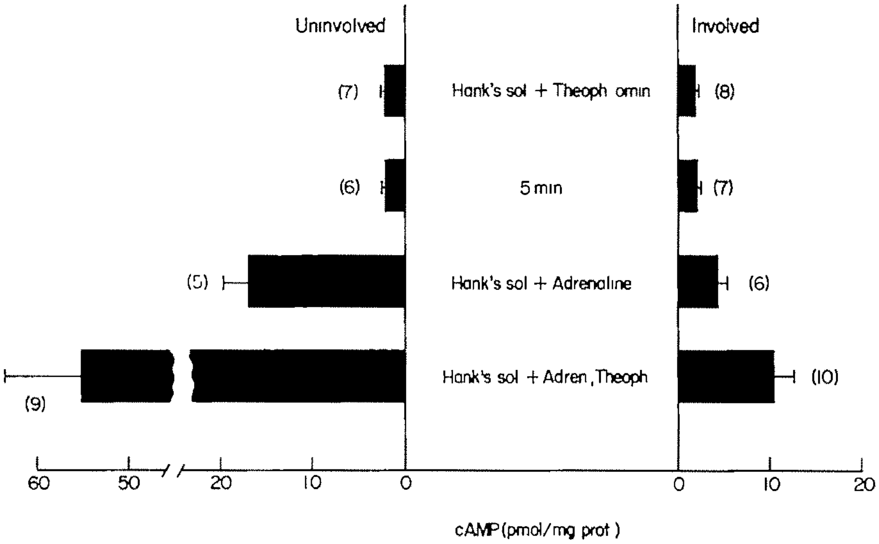

FIGURE 3. Effects of adrenaline (and theophylline) on the epidermal cyclic AMP accumulation. The addition of theophylline (2 mM) alone to Hank's medium did not cause a significant increase in the cyclic AMP level. Data on the cyclic AMP level after adrenaline ($4{\cdot}5 \times 10^{-6}$M) stimulation are all for 5 min incubations. ( ) indicates the number of patients studied.

Since adrenaline has the pharmacological properties of both α- and β-agonists, the effects of α- and β-antagonists were also tested (Fig. 4). Again the cyclic AMP level was stimulated 20- to 30-fold in the uninvolved epidermis of the psoriatic patient and in the normal epidermis from normal subjects but only 3-fold in the involved epidermis of psoriasis subjects. In all cases, however, the cyclic AMP increase by adrenaline was counteracted by propranolol (a β-blocker). The addition of priscoline, an α-blocker, caused a slight to moderate increase in the cyclic AMP level which had already been elevated by adrenaline. This variability in priscoline action may be due to the variable contamination with dermis which contains small blood vessels affected by the α-blocker. At present we can conclude at least that adrenaline stimulation of the adenyl cyclase system in both normal and psoriatic epidermis is due to the β-effect but not to the α-effect.

The ability of other catecholamines to increase the cyclic AMP concentration was examined in one other psoriatic case (Fig. 5). Their effectiveness was as follows: isoproterenol ≥ adrenaline > noradrenaline, and the involved epidermis showed markedly reduced response to all three drugs.

DISCUSSION

Adenyl cyclase is an enzyme complex consisting of regulatory (receptor) and catalytic subunits within the cell membrane. Any attempt to purify this enzyme complex has led to the loss of hormone sensitivity. Therefore the method chosen for this study was to measure the changes in the cyclic AMP level in an epidermal sheet incubated in a physiological medium after the addition of adrenaline. Using a nearly identical system, Voorhees *et al.* (1973) examined whether the response to isoproterenol in the absence of theophylline was abnormal. They found a 117% increase in cyclic AMP concentration after 3 min in the uninvolved as compared to a 36% increase in the involved epidermis. However the baseline level of cyclic AMP in the involved specimens had increased during the 10 min preincubation period and they therefore discarded the difference as being not significant. In addition, the

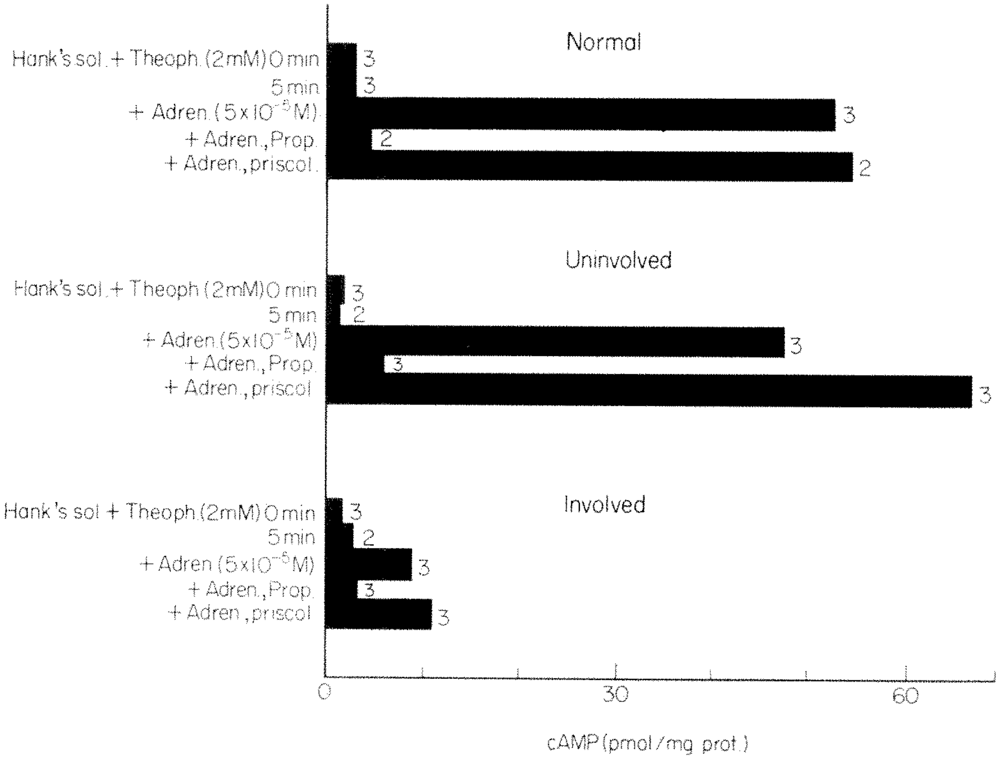

FIGURE 4. Effects of α- and β-blockers on the adrenaline stimulation of epidermal adenyl cyclase. Abbreviations: Adren. = adrenaline; Prop. = propranolol, a β-blocker (5×10^{-4}M); and Priscol. = priscoline, an α-blocker (5×10^{-4}M). Data on the effects of α- and β-blockers were also for 5 min of incubation. The cyclic AMP levels in normal epidermis (from normal subjects) are taken from the accompanying paper by Yoshikawa *et al.* (1975) for comparison.

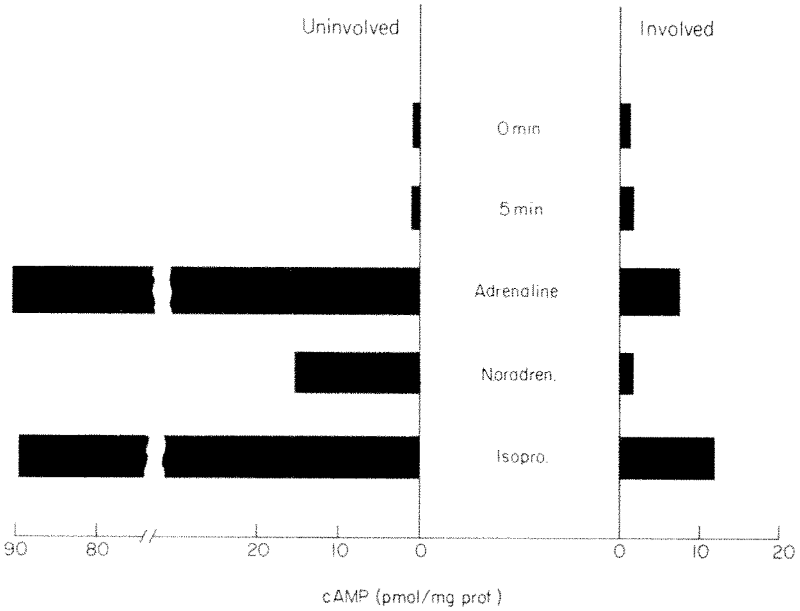

FIGURE 5. Effects of β-agonists on epidermal cyclic AMP accumulation. The concentrations of the agonists were all 5×10^{-5}M. The incubation time was for 5 min. Noradren. = noradrenaline; Isoprot. = isoproterenol.

maximum stimulation was only 100% and thus their preparation was insufficiently sensitive to catecholamines.

Our data do agree with those of Wright *et al.* (1973). Their work was based on the prelabelling with radioactive adenine of the ATP pool in epidermal slices, and after the prelabelling the addition of the hormone (plus theophylline) to be tested. The advantage of this method is that the dynamics of the new cyclic AMP synthesis can be examined, but the interpretation of the data often becomes difficult. The major problem in this method is the uncertainty in establishing the radioactive ATP pool. To solve this problem, Mui, Hsia & Halprin (1975) redesigned these experiments to measure both the ATP pools and the cyclic AMP levels in normal and psoriatic epidermis. Their newer data indicate a clearly deficient response in the involved psoriatic epidermis.

Thus far, our data strongly suggest that hormonal regulation of the adenyl cyclase system in psoriatic epidermis is defective. Since adenyl cyclase activity in psoriatic epidermal homogenate appears to be only slightly lower than that in healthy epidermal homogenate, and also since both psoriatic and healthy epidermal homogenates respond well to NaF (Härkönen, Hopsu-Havu & Raji, 1974), the catalytic subunit itself may be normal in psoriatic epidermis and the possibility of a pathological lesion at the β-receptor site may be indicated. Because the β-blocker suppresses the adrenaline action in both normal and psoriatic epidermis, the adrenaline binding to the receptor sites appears normal in both uninvolved and involved epidermis. The fact that the Kms for adrenaline stimulation of adenyl cyclase in the uninvolved and involved epidermis are about the same suggests that adrenaline has a similar affinity for the binding sites in both uninvolved and involved epidermis. We are then left with the possibility that the total number of binding sites for adrenaline is markedly reduced in the involved epidermis. This possibility appears to be supported by the finding that the V_{max} for adrenaline stimulation is much higher in the uninvolved than in the involved epidermis.

Finally there is no evidence at all bearing on the question of whether or not the poor epidermal adenyl cyclase response is specific to psoriasis. It may of course be a concomitant of any rapidly proliferating epithelium.

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Viral warts, herpes simplex and herpes zoster in patients with secondary immune deficiencies and neoplasms

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SUMMARY

The incidence of viral warts, recurrent herpes simplex and herpes zoster has been assessed in a group of patients with possible secondary immune deficiency states and compared to a control group. Patients with cell-mediated immune deficiency appear to be more prone to warts and zoster infection as compared to patients with humoral immune deficiency. Recurrent herpes simplex infection was not increased in either group.

The central aim of the present study was to determine whether there is a difference in the incidence of viral warts in patients with cell-mediated immune deficiency as compared to patients with humoral immune deficiency. If there is a difference, this may give an indication as to which immunological mechanism is important in controlling this infection. It was felt that it would be interesting to compare and contrast the incidence of recurrent herpes simplex and of herpes zoster in the same patients as, particularly in the case of zoster, an association with immune deficiency has long been known. A report (Hoon & Ansfield, 1964) that viral warts are less common in patients with systemic malignancy prompted the third part of the study; the assessment of the incidence of these three viral infections in patients with cutaneous and systemic neoplasms. The incidence of warts, herpes simplex and zoster was also assessed in a control group to establish a baseline for comparison.

Selection of patients

The main problem in a study such as this is to find a sufficiently large group of patients who are immune deficient. Congenital immune deficiency states were not available in sufficient numbers and therefore the secondary immune deficiency states had to be used. No attempt was made to investigate the immune function of the patients in this study although only, patients with a definite histological diagnosis of the underlying disorder were included.

The disease categories selected were: multiple myeloma as an example of humoral immune deficiency; Hodgkin's disease as an example of cell-mediated immune deficiency; chronic lymphatic leukaemia (C.L.L.) as a disease showing features of both forms of immune deficiency. In addition patients with malignant lymphomas were examined although it was realized that any immune defect in this heterogeneous group is poorly defined; however most studies so far have indicated a cell-mediated immune deficiency, at least in some patients, with malignant lymphomas. In all these conditions, chemotherapy and radiotherapy superimpose a further immune defect. There is such a wide variety of drugs and doses of radio-therapy used, largely with unexplored effects on immunity, that it was felt that this influence would be impossible to control. Instead patients were questioned as to the time relationship of infection to treatment, and time intervals, as documented in case notes, were taken into account.

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MATERIALS AND METHODS

A group of 633 patients and 348 control subjects were questioned and examined; their diagnoses, age and disease duration are shown in Table 1. The group of patients listed as systemic malignancy consisted of carcinomas and sarcomas of various types. The control subjects were seen in a hospital casualty department. They were selected in so far as they were casualties, patients with significant systemic disease being excluded. All patients and controls were over the age of 17 years as inclusion of children would lead to confusing results, particularly in respect of viral warts.

TABLE 1. Details of patients and controls

	Multiple myeloma	Hodgkin's disease	Malignant lymphoma	C.L.L.	Systemic malignancy	B.C.C.	Controls
Number	78	159	109	51	156	80	348
Age	60	35	54	63	56	60	44
Disease duration	3	4	4	4	2.5	2	

B.C.C., basal cell carcinoma of the skin; C.L.L., chronic lymphatic leukaemia.

Each person was personally interviewed by the author and details recorded on a standard question sheet. It was first established that the individual understood what the terms, wart or verruca, herpes simplex or cold sores, herpes zoster or shingles, meant, and then details regarding the infections were sought. Lesions stated to be warts were examined, as well as any other lesion mentioned by the patient. Subjects not having an adequate understanding of the English language or who were unable to comprehend the questions due to age or illness were excluded. The term herpes simplex was used mainly in reference to cold sores and no attempt was made to ascertain the incidence of herpes simplex in other areas of the body.

RESULTS

Present viral warts

The results are shown in Table 2. It can be seen that patients with Hodgkin's disease, malignant lymphoma or chronic lymphatic leukaemia have a considerably increased incidence of warts. The average number of warts as compared to controls is also increased, and in the majority of affected patients with these conditions, the warts preceded the onset of symptoms from the underlying disease. Four patients with Hodgkin's disease, one with lymphoma and one with C.L.L. had had warts successfully removed since the onset of their disease. However, although over 50% of the patients with

TABLE 2. Incidence of present viral wart infections

	Incidence (%)	Incidence before treatment (%)	Mean number of warts
Control subjects	2·3		1
Multiple myeloma	0		
Hodgkin's disease	29 6	22	6
Malignant lymphoma	20·1	12	16
C.L.L.	17·6	8	16
Systemic malignancy	4·5		8
B.C.C.	6·2		1

warts were receiving treatment for their warts, no others had had any success in eradicating them. Five patients related the appearance of their warts to chemotherapy or radiotherapy, while two had noted considerable reduction in the number of their warts with chemotherapy.

The site and type of warts were noted. No particular localization was found although, surprisingly, no cases of ano-genital warts were seen in this survey. Plane warts were by far the most common type in patients with Hodgkin's–lymphoma–leukaemia, particularly in those having a great number of warts.

Past viral warts

There were no outstanding differences in the various conditions either over the whole of the lifetime of the patients or after the age of 20 years.

Recurrent herpes simplex

The incidence of this infection is given in Table 3. It can be seen that patients with multiple myeloma and systemic malignancy have a much lower incidence as compared to both the control group and patients with other neoplasms. There is a predominance of males, particularly in the control group. The frequency of recurrences of herpes simplex was also recorded and patients were asked whether they thought that attacks were more or less frequent over the past 5–10 years. Nineteen patients with a malignancy or lymphoma had noted an increased frequency of attacks since diagnosis of their underlying disease and all related the increase to treatment.

TABLE 3. Incidence of herpes simplex infection

	Incidence (%)	Male/female
Control subjects	39	102/34
Multiple myeloma	21	12/4
Hodgkin's disease	39	36/23
Malignant lymphoma	42	26/20
C.L.L.	39	14/6
Systemic malignancy	23	20/16
B.C.C.	35	16/12

Herpes zoster

The incidence of this infection is given in Table 4. Herpes zoster is more common in patients with Hodgkin's disease, malignant lymphoma and C.L.L. as compared to the other groups and controls. The increased incidence is mainly accounted for by attacks occurring after the onset of the disease. It is interesting that five patients with lymphomas gave a history of two separate episodes of zoster infection.

TABLE 4. Incidence of herpes zoster infection

	Incidence (%)	Cases occurring after onset of disease (%)
Controls	11	
Multiple myeloma	15	8
Hodgkin's disease	28	68
Malignant lymphoma	26	46
C.L.L.	28	64
Systemic malignancy	13	30
B.C.C.	15	8

DISCUSSION

Viral warts

This survey shows a greatly increased incidence of warts in patients with Hodgkin's disease and a lesser but still marked increase in patients with C.L.L. and malignant lymphoma. As explained earlier, this group are at least to some degree deficient in cell-mediated immunity. In contrast, patients with multiple myeloma do not show an increased incidence of warts and these patients are, if anything, deficient in humoral antibody responses. The increase of warts in the Hodgkin's–lymphoma–leukaemia group is not just due to treatment because the incidence had risen before any treatment had been given and, furthermore, myeloma patients also receive chemotherapy and radiotherapy.

The results of this survey suggest that patients with cell-mediated immune deficiency are prone to wart infection while those with humoral immune deficiency are not. Furthermore, once a patient with such a deficiency develops warts they are unlikely to resolve spontaneously or to show a satisfactory response to conventional therapy. It is therefore suggested that cell-mediated immune responses are an important factor both in preventing warts and in bringing about their successful resolution. In addition it is suggested that humoral antibodies are not important in preventing wart infection. Only one previous survey of warts in immune deficient patients could be found in the literature (Spencer & Anderson, 1970). They found that 42% of fifty-five renal transplant patients developed warts within a year of transplantation. Prednisone and azathioprine, used for immunosuppression in their patients, particularly depresses cell-mediated immunity and therefore that study would appear to support the present results.

Patients with systemic and cutaneous malignancy of the carcinoma–sarcoma variety do not appear to be different from the control group so far as the incidence of warts is concerned. Therefore this survey does not support the suggestion of a reduced incidence of warts in patients with carcinoma (Hoon & Ansfield, 1964).

Recurrent herpes simplex

Two interesting findings emerge from this part of the study. Firstly, the low incidence of herpes simplex found in patients with myeloma and systemic malignancy. A recent survey (Ross & Tyrrell, 1974) among patients with carcinoma of the lung found a very similar result, 21% of patients having a history of herpes simplex infection as against 42% of the controls. The authors suggested that recurrent herpes simplex raises the level of immunity to malignancy by stimulating immunological surveillance. However if this was the case the Hodgkin's–lymphoma–leukaemia group, which are also malignancies, might be expected to show a reduced incidence, but in the present study they differ little from the controls.

The second interesting finding is that there is no indication from this survey that immunological factors are important in determining the incidence of recurrent herpes simplex. The role of immune function in this infection in humans is at present poorly defined. That humoral antibodies alone play no significant part has been established (Douglas & Couch, 1970) but the importance of cell-mediated immunity is debatable. Severe herpes simplex has been reported in renal transplant patients (Montgomerie *et al.*, 1969) but the four patients discussed also had cytomegalovirus in their lesions, a virus now known to cause severe infections in immuno-suppressed patients (Lopez *et al.*, 1973). The Wiskott–Aldrich syndrome is another disease reported (Cooper *et al.*, 1968) to show an increased incidence and severity of herpes simplex infection. However patients with this syndrome also have eczema, which can of course lead to widespread herpes simplex infection in the absence of any demonstrable immune defect. Two surveys (Spencer & Anderson, 1970; Rifkind, 1964) as opposed to case reports, have found that the incidence and severity of recurrent herpes simplex is not increased in immuno-suppressed renal transplant patients.

An increase in frequency of attacks was noted in all disease groups by at least some patients in the present study and was usually attributed to treatment. This increase is probably not due to an immune disturbance, but instead, could arise from non-specific factors such as debility, operating in a similar manner to coryza and sunlight, both well-known precipitants of herpes simplex infection.

Herpes zoster

The association of herpes zoster and malignancy has long been recognized and has been analysed in many different ways, but this appears to be the first survey to look at the incidence from the viewpoint of the immune state of the patients. There is a considerable increase in the incidence of zoster in the Hodgkin's–lymphoma–leukaemia groups and this increase is mainly accounted for by cases occurring after the onset of the disease. Therefore treatment may have an influence here. However myeloma patients also receive similar immuno-suppressive treatment and they do not show this increase after the onset of the disease. In fact, even though the myeloma patients are in an older age group than the controls, they show little increase in the incidence of zoster infection.

As with warts, the present survey showed that the most marked increase in the incidence of zoster occurs in patients with some degree of cell-mediated immune deficiency while those having only a humoral immune deficiency show little increase. It is therefore possible that the prevention of herpes zoster is a function of cell-mediated immune responses.

CONCLUSIONS

(1) The incidence of viral warts is increased in patients with:

Hodgkin's disease
Malignant lymphoma
Chronic lymphatic leukaemia

(2) The incidence of herpes zoster is increased in patients with:
 Hodgkin's disease
 Malignant lymphoma
 Chronic lymphatic leukaemia

(3) This study suggests that:
 (a) Cell-mediated immunity may be important in both the prevention and resolution of infections due to wart virus.
 (b) Cell-mediated immunity may be important in preventing infections due to varicella–zoster virus.
 (c) Immunological factors are not important in preventing recurrent herpes simplex infections.

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Epidermal antigens in lichen planus

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SUMMARY

The loss of intercellular and basement membrane antigens has been associated with the development of epidermal cell dysplasia and neoplasia. The antigenic components of the epidermis have been investigated in lichen planus, lupus erythematosus and eczema, using pemphigoid and pemphigus sera by the indirect immunofluorescent staining method. In areas of active lichen planus there was extensive reduction or complete absence of basement membrane antigen. Intercellular antigen was preserved in lichen planus although in some damaged rete ridges it appeared to be reduced. Both these antigens were well preserved in lupus erythematosus and eczema. Loss of epithelial antigens is therefore not confined to the development of neoplasia.

Using the indirect immunofluorescent (IF) staining technique the majority of patients with pemphigus and pemphigoid can be shown to have circulating antibodies to the intercellular and basement membrane areas, respectively, of mammalian stratified squamous epithelium. The antibodies of these bullous diseases have been employed to detect alteration of epidermal antigenicity occurring in human skin cancer (De Moragas, Winkelmann & Jordan, 1970 a & b) and also in experimentally induced skin dysplasia and neoplasia in mice (Muller & Sutherland, 1971). Both these studies indicated that intercellular (IC) antigen was apparently diminished or lost with increasing dysplasia of the epidermal cells. Basement membrane (BM) antigen staining was also reduced as the cell became more dysplastic; and particularly in association with deep invasive squamous cell carcinoma might be completely absent. Similar studies on squamous cell neoplasms of non-cutaneous origin, however, showed that the majority of both primary and secondary tumours retained IC and BM antigen staining (Pertschuk & Rosen, 1973) but the reason for this disparity was not clear.

The same methods have been used to study the development of epidermal antigens in the fetus (Muller, Kalnins & Sutherland, 1973; Delpech-Stewart, 1971) and also the regeneration of the basement membrane region under migrating epidermal cells in an experimental *in vitro* model of wound healing (Marks, Abell & Nishikawa, 1975).

Although the alteration or loss of epidermal antigens has been shown to be associated with cellular dysplasia, no study appears to have been undertaken to examine the changes produced by inflammatory reactions. While examining a series of biopsies of lichen planus papules by direct IF staining it was considered that such a study might prove of interest, particularly in this condition in which the inflammatory changes are directed against the basal epidermal cells. Moreover, ultrastructural studies of lichen planus have shown that the basal lamina may be disrupted, lost or even reduplicated (Brody, 1969; Johnson & Fry, 1967; Ebner & Gebhart, 1972; Sarkany & Gaylarde, 1971), these changes presumably being brought about by the continued process of destruction and repair of this region which is thought to characterize the disease process.

MATERIALS AND METHOD

Elliptical skin biopsies were obtained from nineteen cases of active lichen planus (LP), five cases of lupus erythematosus (LE), and seven cases of eczema. The specimens were divided, half being rapidly frozen in liquid nitrogen and stored at −70°C, and half being fixed in formal-saline 10% for routine histological processing.

The frozen material was sectioned at 5 μm at −25°C within 1 week of collection, and stained by the indirect method as follows. After preliminary washing in phosphate buffered saline, sections were incubated in moist chambers at 37°C, with either normal human serum, pemphigoid, or pemphigus serum at 1:10 and 1:80 dilutions, for 30 min. After further washing, a second incubation was performed using a commercially obtained sheep antihuman IgG (Wellcome reagents) F/P ratio 4·1 molar, Ab/P 0·71 mg/ml, 4 units per ml, diluted to 1:30. The sections were mounted in buffered glycerol at pH 8 and examined in a Leitz SM fluorescent microscope, illuminated by a HBO 200 lamp, using a BG 38 (red) and a KP 490 FITC primary filter, and secondary filters 510 or 530.

Sera from two cases of pemphigoid and two cases of pemphigus were used in these experiments. Each serum had a titre of at least 1:640 against normal human skin. Normal skin sections, stained in the same way, were incorporated as controls in all experiments. Histological confirmation of all diagnoses was obtained from the paraffin processed haematoxylin and eosin stained tissue. All biopsies included areas of uninvolved skin adjacent to the abnormal sites, except in the biopsies of eczematous skin.

All nineteen cases of LP demonstrated globular or cytoid body deposits of immunoglobulins (mainly IgM) together with fibrin deposition in the papillary dermis, on direct IF staining. The five cases of LE were chosen because of the absence of IgG in the IF basement membrane band. IgM and IgA were demonstrated together with complement in the BM zones of three of these biopsies, and two had negative IF findings. All five had characteristic histological findings of LE, and four showed cytoid body deposits of IgM in the dermal papillae. Two of the five eczema cases had the histology of lichenification, and the rest were of the subacute type.

RESULTS

(a) *Basement membrane antigens*

Continuous linear BM fluorescent staining was demonstrated in all the sections of normal skin, lupus erythematosus, and eczema. Only in two biopsies of subacute eczema were small breaks in the basement membrane seen.

In the uninvolved areas of all the LP sections this same linear fluorescent band was preserved, but in the LP papules all demonstrated diminution of BM staining. Breaks in the BM were readily apparent in eighteen of these papules and were of variable extent. The most frequent pattern was of multiple small breaks or short gaps in areas of obvious LP activity but occasionally the BM was almost completely absent over a whole rete ridge (Fig. 1).

No other specific staining was seen other than at the epidermo-dermal junction. Hyaline, or IF cytoid bodies were not visualized.

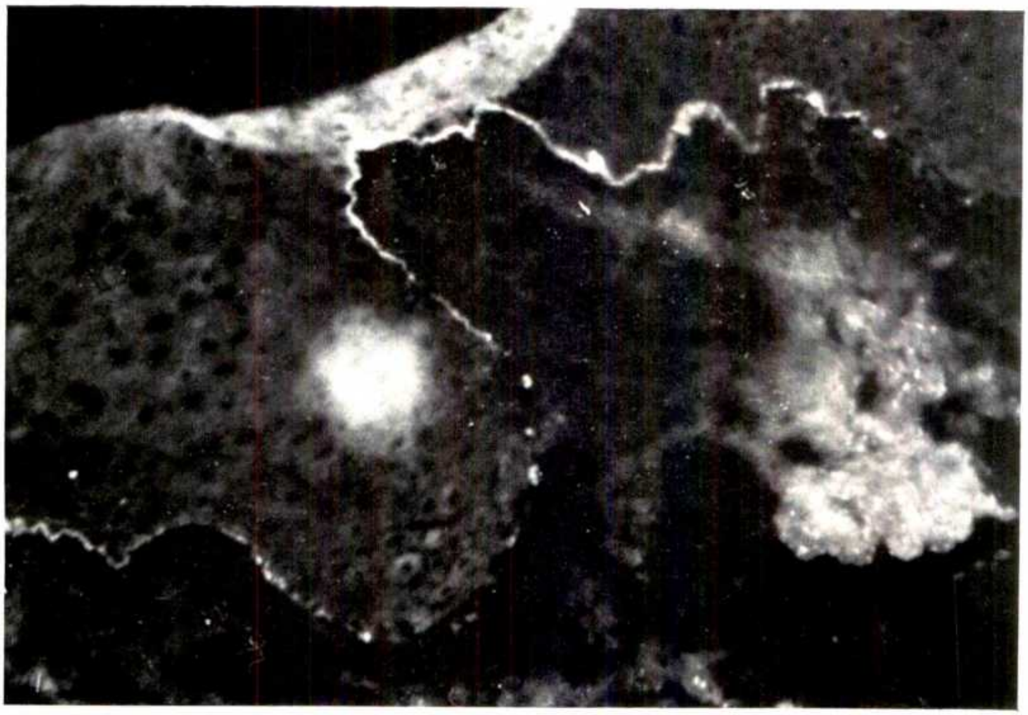

FIGURE 1. Indirect IF of LP with pemphigoid serum. BM staining is almost completely absent from rete ridge (× 125).

(b) *Intercellular antigens*

The antigenicity of the intercellular area was preserved in all the specimens, whether from normal skin, LE, eczema, or LP. As is the usual pattern, IC staining tended to be weaker around the basal cells, and this was also the case in the LE and LP biopsies. In four biopsy specimens of LP, however, the IC staining pattern was reduced at the tips of some saw-toothed rete ridges (Fig. 2).

Hyaline or cytoid IF bodies were not demonstrated by this method.

DISCUSSION

Extensive deficiency of stainable BM antigen in these experiments was confined to LP papules. The short breaks seen in two biopsies of subacute eczema were of a localized type and were not associated with the marked reduction of BM staining in the adjacent areas seen in LP lesions.

Despite the similarity of histological localization of tissue damage in LP and LE, the latter condition failed to show evidence of alteration or destruction of BM antigens. These initial experiments on LE were performed upon cases failing to show IgG deposition at the BM zone on direct IF staining. IgG, deposited at this site in LE biopsies, interferes with the demonstration of BM antigens by this indirect method. Prior elution of this IgG antibody would probably enable all LE lesions to be used, but this does introduce an unnecessary additional reaction to the method.

It has been suggested that the primary change in LP is that of basal epidermal cell degeneration (Thyresson & Moberger, 1957) and that the areas sustaining the damage are repopulated by migration

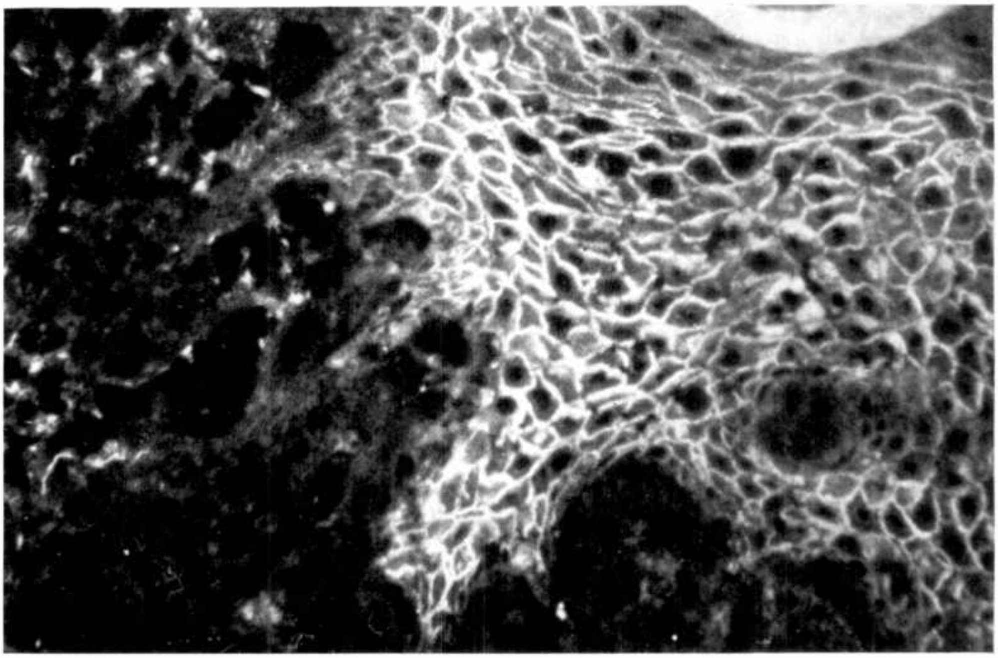

FIGURE 2. Indirect IF of LP with pemphigus serum. The IC staining is preserved in this active papule but is much reduced in the rete ridges on the left of the micrograph (× 300).

of cells from the viable margins (Marks, Black & Wilson Jones, 1973; Presbury & Marks, 1974). There is some evidence to suggest that the basal cells elaborate basal lamina (Hay & Dodson, 1973) and possibly also basement membrane antigen (Marks *et al.* 1975) although these antigens have not been definitely shown to reside within the basal lamina.

If the cells repopulating the lichen planus papule are responsible for the reduplication of the basal lamina seen by electron microscopy, they cannot be shown by this IF method to have elaborated BM antigens. However, it is probable that any newly formed BM antigen would be destroyed in the same way as the original material by the continued LP process.

These experiments clearly demonstrate that BM antigen can be structurally altered or lost in at least one inflammatory dermatosis, as well as in association with epidermal cell dysplasia. At least two possible mechanisms might be responsible for these changes in LP. BM antigen may be damaged by the inflammatory cellular reaction or alternatively the damage to the epidermal basal cells may be sufficient to prevent them elaborating BM antigenic material. Considering the preservation of the BM fluorescent staining in eczema and LE it would seem that the latter process is the more likely.

The hyaline or Civatte body in LP (and LE) is derived from the degeneration of basal epidermal cells (Thyresson & Moberger, 1957; Ueki, 1969; El-Labban, 1970) possibly during mitotic division (El-Labban & Kramer, 1974). These bodies are probably the equivalent of the cytoid or globular deposits seen on direct IF staining (Ueki, 1969; Michel & Sy, 1973; Baart de la Faille-Kuyper & Baart de la Faille, 1974; Abell *et al.* 1975). These bodies do not appear to retain the IC antigen, nor do they bind BM antibody, as might be expected if, as suggested by Ueki, some of these bodies (especially in LE) were derived from thickened basement membrane.

The epidermal cells in LP, as well as in other dermatoses, are able to elaborate IC antigen. This antigen probably resides in the glycocalyx (Hashimoto *et al.*, 1974) and this extracellular material appears to play a part in cellular cohesion. In a few sections the saw-toothed rete ridges, although showing diminished IC staining, did not lose this material completely. In severe LP reactions, clefts or frank bullae may develop in the basal cell layer, and it is interesting to speculate that the absence of histological acantholysis in such damaged epidermis is due to the preservation of this intercellular material.

Ultrastructural studies of LE have not demonstrated loss of basal lamina although this has been described in lichen sclerosus et atrophicus (Mann & Cowan, 1973), bullous pemphigoid (Schaumburg-Lever, Orfanos & Lever, 1972), dermatitis herpetiformis (Fry & Johnson, 1969) and cicatrical pemphigoid (Caputo, Bellone & Crosti, 1973).

More extensive testing of inflammatory dermatoses is required to determine whether the loss of BM antigen staining is confined to LP and also whether this reaction is shared by the other lichenoid conditions.

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Light and electron microscopic differentiation of amyloid and colloid or hyaline bodies*

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SUMMARY

Hyaline or colloid bodies (Civatte bodies) and amyloid are sometimes confused because of their similar appearance under the light microscope. A differentiation can be made by histochemical means (DMAB-Nitrite method) and by fluorescence or polarization microscopy.

At the ultrastructural level hyaline or colloid bodies as well as amyloid exhibit a fibrillar structure, but can be differentiated by the diameter and arrangement of fibrils and the localization of the aggregations. A clearcut differentiation between hyaline or colloid bodies and amyloid seems of great importance in any discussion of the epidermal origin of amyloid and the classification of fibrillar aggregations associated with skin tumours.

Round or ovoid, amorphous, eosinophilic masses can be observed in the region of the dermo-epidermal junction in normal human skin as well as in pathological conditions. One group of these formations is known in histopathology as colloid or hyaline bodies (Civatte-bodies) and at present it seems likely that they derive from degenerating epidermal cells (Anton-Lamprecht & Tilgen, 1972, 1973; Ebner & Gebhart, 1972a, 1972b; El-Labban, 1970; El-Labban & Kramer, 1974). Their fibrillar ultrastructure indicates a tonofilament origin and therefore it is assumed that Civatte bodies represent epidermal material which is eliminated into the corium (Anton-Lamprecht & Tilgen 1973; Ebner & Gebhart, 1972a; El-Labban, 1970; El-Labban & Kramer, 1974).

Using the light microscope, Black (1971) and Black & Wilson Jones (1971) pointed out that these hyaline or colloid bodies are similar in size and appearance to the small amyloid deposits within the dermal papillae frequently seen in macular amyloidosis and lichen amyloidosus. Also, when examined with the electron microscope, great similarities exist between amyloid and Civatte bodies because of their fibrillar ultrastructure (Anton-Lamprecht & Tilgen, 1973). A clearcut ultrastructural differentiation of these structures seems of great importance especially in the discussion of the epidermal origin of amyloid (Black, 1971; Black & Wilson Jones, 1971; Black & Heather, 1972) and the classification of fibrillar aggregations associated with skin tumours (Hashimoto & King, 1973; Hashimoto &

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Brownstein, 1973). The aim of the present study is to differentiate between hyaline or colloid bodies and amyloid with the light and electron microscope.

MATERIALS AND METHODS

Five patients, one with lichen amyloidosus (LA) and four with active papular lichen planus (LP),

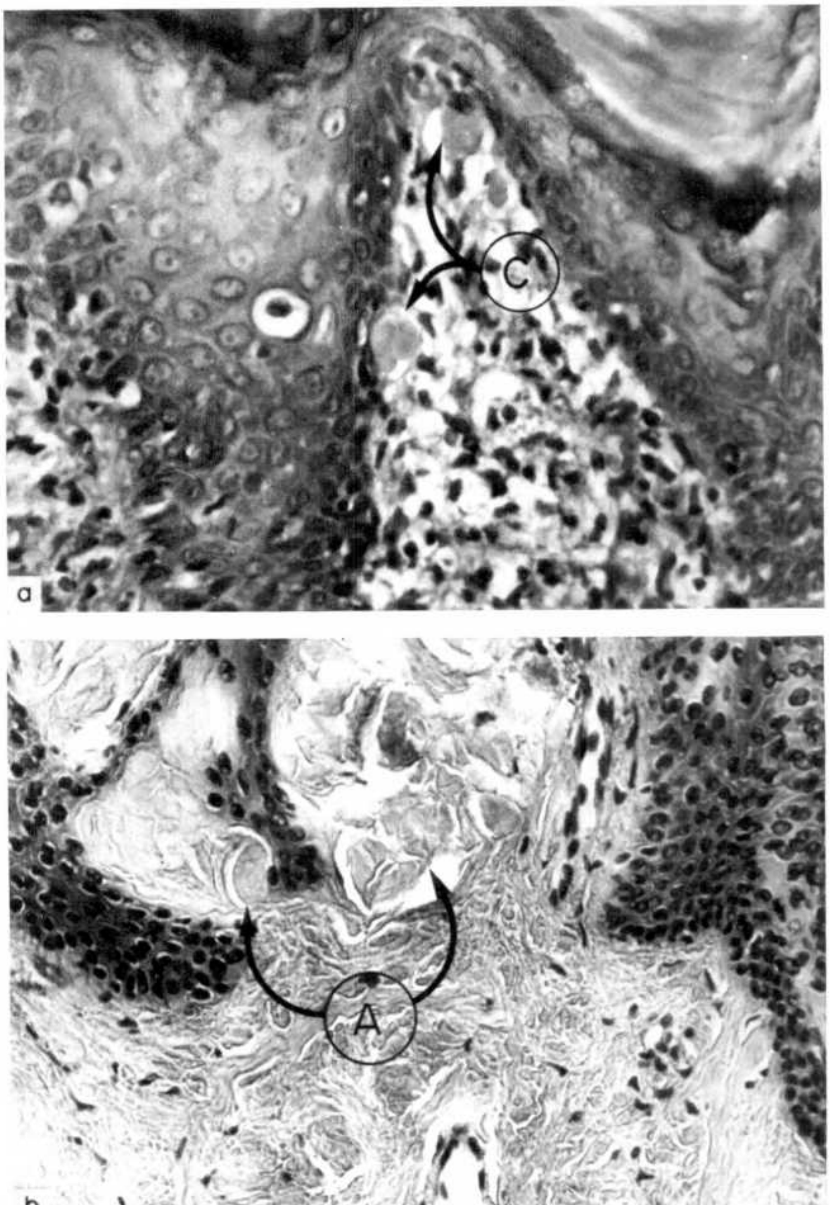


FIGURE 1. Haematoxylin and eosin. (a) Lichen planus. Several Civatte bodies at the dermo-epidermal junction are indicated by arrows. ×450. (b) Ovoid amyloid deposits in the dermal papillae resembling Civatte bodies. ×350.

were studied. Punch biopsies were taken from clinically affected skin under local procaine anaesthesia.

The specimens were cut into halves.

(1) One half was fixed in formalin and then embedded in paraffin. The following stains were used: Haematoxylin and eosin; Trichrome (van Gieson and Goldner) Weigert's elastica stain; PAS

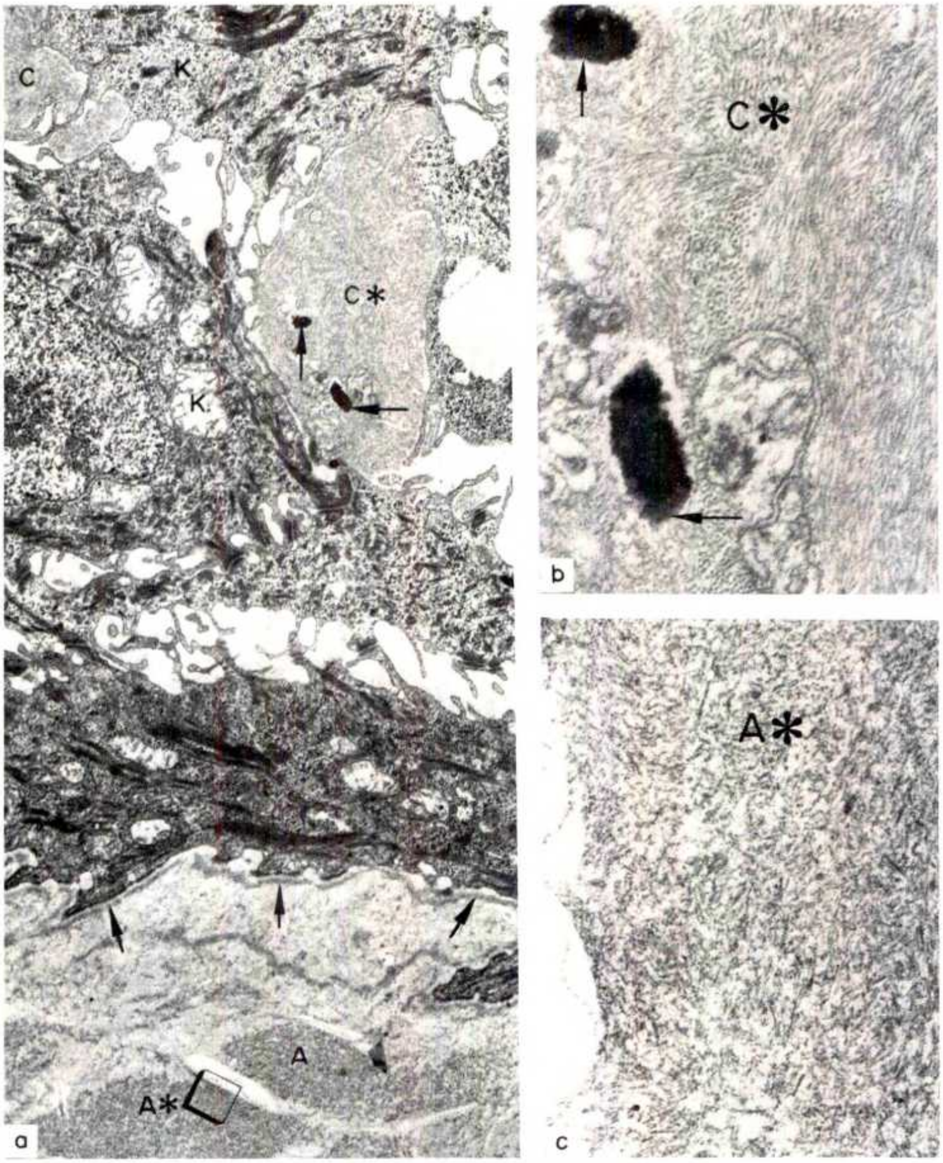


FIGURE 2. (a) Lichen amyloidosus. Intraepidermal Civatte bodies (C) containing pigment granules and subepidermal amyloid deposits (A) are present. The basal lamina is marked by spikes. ×15,000. (b) Detail of the Civatte body (C*). The weavy or whorl-like arrangement of fibrils is clearly visible. ×65,000. (c) Detail of dermal amyloid showing the irregular arrangement of fibrils. ×35,000.

(McManus) before and after diastase; DMAB Nitrite (Adams); Thioflavine-S; toluidine blue at pH 5 and 7; Feulgen reaction. In addition paraffin sections stained with Congo red were investigated by polarization microscopy.

(2) The other half of the specimen was immediately fixed in 3% phosphate buffered glutaraldehyde (pH 7·4), postfixed after thorough buffer washes in 1% osmic acid, dehydrated with graded concentrations of ethanol, and embedded in Epon. Thin sections were cut with diamond knives, using a Reichert Ultramicrotome (Type OMU 3) and stained with uranyl acetate and lead citrate. Sections were examined under a Zeiss electron microscope (EM 9 s/2).

The material investigated and published in previous papers dealing with amyloidosis of the skin (Ebner, 1968) and Civatte bodies (Ebner & Gebhart, 1972a, 1972b) was also included in this study.

RESULTS

(1) *Light microscopy*

A variable number of amorphous, eosinophilic round or ovoid bodies was present at the dermo-epidermal junction of all cases of LP. They were 10–25 μm in size and situated mostly within or above the inflammatory cell infiltrate typical of this condition (Fig. 1a). Sometimes they were found also intra-epidermally. In some areas the hyaline or colloid bodies were accumulated in dermal papillae. Very often they were separated from the surrounding connective tissue by cleft formations probably representing artefacts due to fixation.

In LA the amyloid deposits were situated immediately beneath the epidermis and sometimes tended to invade the epithelium, thus disrupting the PAS-positive basement membrane. In haematoxylin and eosin stained preparations amyloid has a homogeneous, acidophilic appearance (Fig. 1b). Usually the deposits are larger than the hyaline or colloid bodies described above. However in distinct areas, especially at the periphery of the deposits, there are globes of amyloid closely resembling the Civatte bodies. Amyloid and hyaline or colloid bodies show identical properties with haematoxylin and eosin, van Gieson and PAS stains. The light microscopic differences are listed in Table 1.

TABLE 1. Light microscopic staining properties of amyloid and hyaline or colloid bodies (Civatte bodies)

Staining method	Amyloid	Hyaline or colloid bodies
Haematoxylin-eosin	Pink	Pink
Trichrome Goldner	Green	Yellow
Trichrome van Gieson	Yellow	Yellow
PAS-McManus	Purple	Purple
PAS after diastase pretreatment	Purple	Purple
DMAB-Nitrite Adams	Blue	Unstained
Thioflavine S	Yellow fluorescence	No fluorescence
Toluidine blue (pH 5 and 7)	Lilac	Blue-green
Feulgen reaction	Negative	Negative
Congo red (polarization microscope)	Green	Negative

(2) *Electron microscopy*

The electron microscope reveals the fibrillar ultrastructure of hyaline or colloid bodies (Figs. 2b, 3a, 4a). They are composed of bundles consisting of tightly packed fine filaments, 80–100 Å wide. Because of an orthogonal arrangement of these fibrillar masses the filaments can be seen cut

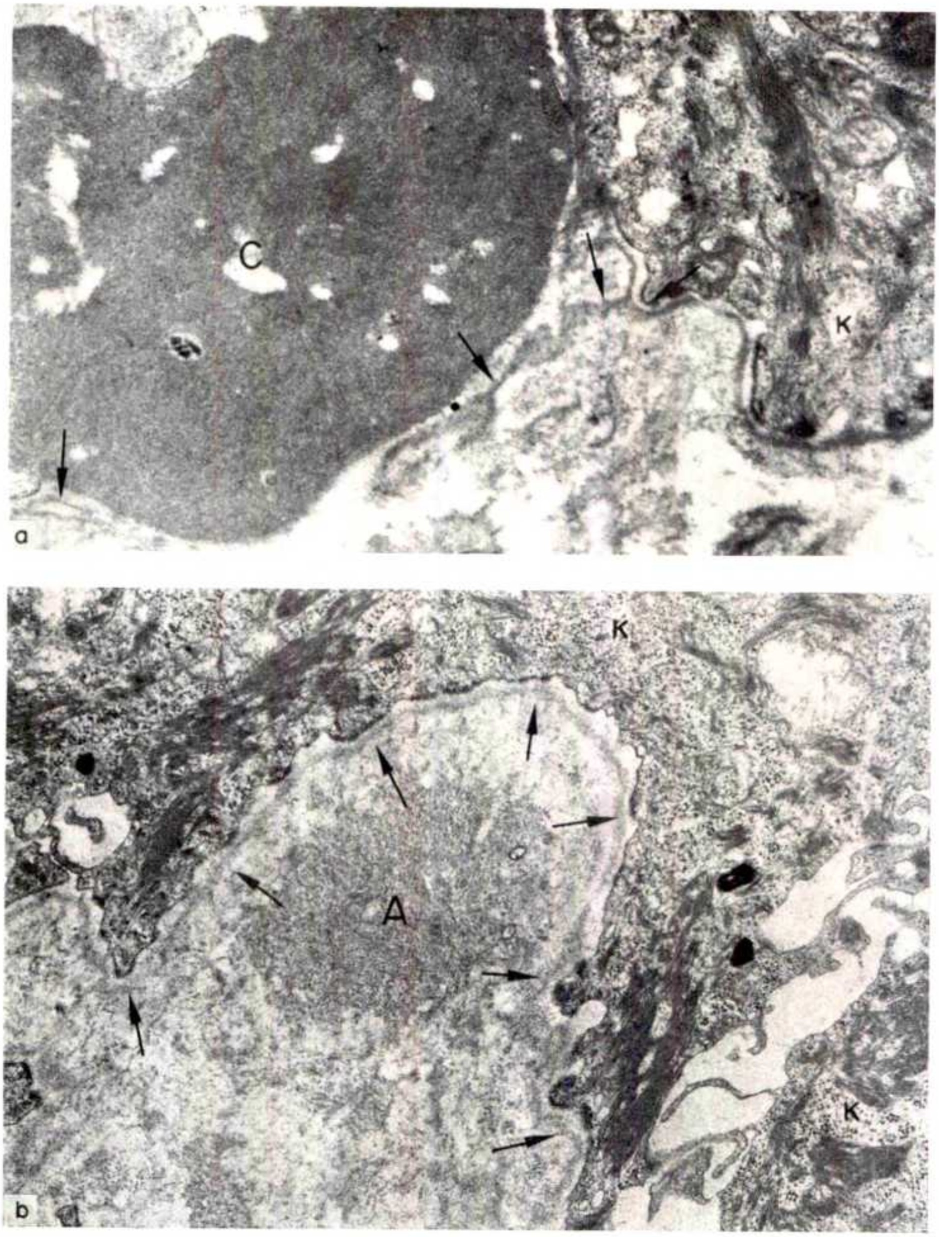


FIGURE 3. (a) Lichen planus. Civatte body in transition from the epidermis to the dermis bulging the basal lamina (↓) towards the corium. ×30,000. (b) Lichen amyloidosus. Amyloid deposit (A) in uppermost corium bulging the basal lamina (↑) towards the epidermis. ×24,000.

either transversely or longitudinally (Fig. 2b). Hyaline or colloid bodies often have a whorled aspect. Usually they do not possess a limiting membrane, but are often encircled by cytoplasmic processes of fibroblasts. In many instances the hyaline or colloid bodies contain cytoplasmic organelles like melanosomes or mitochondria (Figs. 2a, b, 3a). Until now we could not find any nuclear material.

The same fibrillar bodies are localized in the basal layer of the epidermis and sometimes show a tendency to bulge the basal lamina towards the corium (Fig. 3a).

In LP there were only Civatte bodies but no amyloid deposits, whereas in LA both structures could be found, sometimes even in the same section (Figs. 2a, b, c). Amyloid islands of different size are present immediately beneath the basal lamina (Figs. 2a, 3b). At high magnification amyloid is seen to be composed of filaments measuring 60–80 Å in diameter (Figs. 2c, 4b). These filaments show an irregular arrangement. Only in small areas can an aggregation into bundles be observed. Sometimes

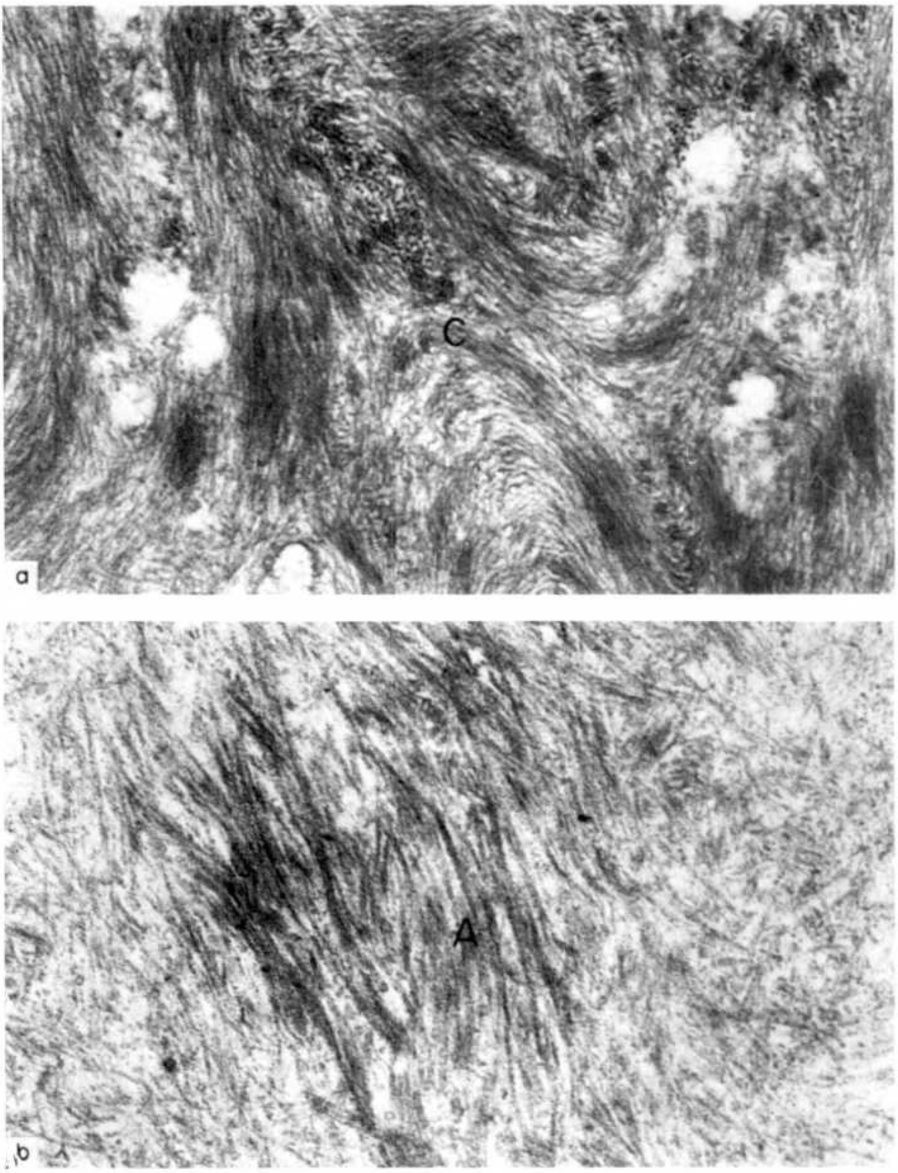


FIGURE 4. (a) Lichen planus. High magnification of Civatte body. ×70,000. (b) Lichen amyloidosus. High magnification of amyloid. ×80,000.

the amyloid fibrils seem to possess a tubular structure. Along the periphery of individual amyloid islands these deposits are often surrounded by fibroblasts, and typical collagen fibrils are present. In circumscribed areas the basal lamina is invaginated or masked by amyloid material (Fig. 3b).

DISCUSSION

The data presented show that a differentiation between amyloid and hyaline or colloid bodies is possible by means of the light and electron microscopes. As pointed out at the beginning, an exact distinction between these fibrillar deposits seems to be of great importance in the investigation of secondary localized cutaneous amyloidosis as well as to throw light upon the question of epidermal amyloid production.

The commonly used term hyaline or colloid bodies has to be seen in a historical context and is not quite satisfactory considering our knowledge about the origin of these structures. The hyaline masses deposited in lipoid-proteinosis (Kint, 1970; Rodermund & Klingmüller, 1970) and the material of colloid milium (Hashimoto, Miller & Bereston, 1972) can be clearly differentiated from Civatte bodies by the electron microscope.

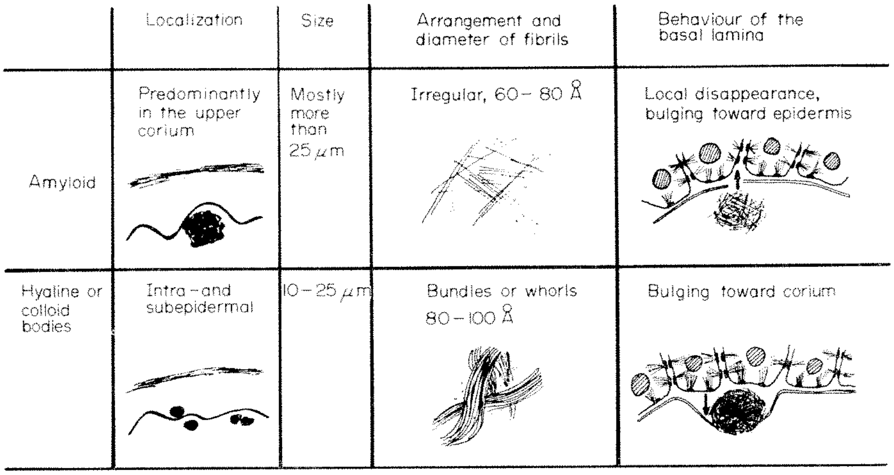
The ultrastructural distinction between colloid bodies and amyloid is much more difficult, since both fibrillar structures are of very similar appearance and both may be located in the dermo–epidermal junction area. These similarities raise the question of the relationships between amyloid and Civatte bodies (Anton-Lamprecht & Tilgen, 1973; Black, 1971). Our investigations of L.A., in which both Civatte bodies and amyloid deposits were present in the same specimen, disclosed that a differentiation between these structures can be made at the ultrastructural level by considering the following parameters:

(a) The localization and the size of the deposits.

(b) The arrangement and the diameter of the filaments. It has to be kept in mind that the size and shape of filaments depends on the processing techniques and can only be compared in tissues fixed, dehydrated and stained in an identical manner.

(c) The relationship to neighbouring structures, e.g. the basal lamina, adjacent keratinocytes or connective tissue cells. The main differences between both structures found in our material are listed in Fig. 5.

Electron microscopically, hyaline or olloid cbodies are fibrillar structures representing tonofilament



	Localization	Size	Arrangement and diameter of fibrils	Behaviour of the basal lamina
Amyloid	Predominantly in the upper corium	Mostly more than 25 μm	Irregular, 60–80 Å	Local disappearance, bulging toward epidermis
Hyaline or colloid bodies	Intra-and subepidermal	10–25 μm	Bundles or whorls 80–100 Å	Bulging toward corium

FIGURE 5. Ultrastructural differences of amyloid and hyaline or colloid bodies (Civatte bodies).

material, which is eliminated into the dermis (Anton-Lamprecht & Tilgen, 1973; Ebner & Gebhart, 1972a). This seems to be a normal mechanism found in different, unrelated conditions and even in unchanged human skin (Ebner & Gebhart, 1972b; Gebhart, Kidd & Niebauer, 1971; Gebhart, Sluga-Gasser & Lassman, 1971; Goltz & Hult, 1963; Grishman & Churg, 1970; Haustein, 1973; Kobayasi & Asboe-Hansen, 1974; Lever, 1967; Pinkus & Mehregan, 1969; Sabouraud, 1910; Stankler & Ewen, 1974; Thyresson & Moberger, 1957; Ueki, 1969). According to Weedon (1974) they could probably represent keratinocytes undergoing 'apoptosis'.

In damaged skin, especially in association with pigmentary incontinence, this reaction can be found more frequently (Anton-Lamprecht & Tilgen, 1973; Ebner & Gebhart, 1972a; Goltz & Hult, 1963; Stankler & Ewen, 1974). El-Labban & Kramer (1974) described Civatte bodies in oral lichen planus which contained nuclear material. In our experience two different structures have to be separated in LP (Ebner & Gebhart, 1972b).

(1) *Fibrillar bodies* which represent the hyaline or colloid bodies (Civatte bodies). They can be found frequently in L.P. but are not pathognomonic for the disease.

(2) *Dyskeratotic keratinocytes* as a morphological sign of early epidermal involvement in LP These cells, although present in some other conditions, seem to be more specific for LP than hyaline or colloid bodies. We doubt whether all these dyskeratotic cells are transformed into hyaline or colloid bodies. Furthermore Civatte bodies can also be found in normal skin and in several conditions in which no dyskeratotic elements are present. Therefore a different mechanism—e.g. a direct transformation of cytoplasmic compartments of keratinocytes—has to be considered.

In our opinion the question of the epidermal contribution to amyloid formation resulted from the fact that in LA and macular amyloidosis increased formation of hyaline or colloid bodies takes place in association with incontinence of pigment. At present exact data concerning the chemical structure of Civatte bodies are lacking, but it is unlikely that the fibrillar tonofilament material of hyaline or colloid bodies could be transformed into amyloid.

At present we have no definite results on the presence of hyaline or colloid bodies in skin tumours, but there is a possibility that this mechanism is operative also in these conditions and should be kept in mind when secondary localized cutaneous amyloidosis is investigated by electron microscopy.

ACKNOWLEDGMENTS

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Heterogeneity of bovine snout epidermal prekeratin

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SUMMARY

The prekeratin of the snout of cows was extracted with 0·1 mol/l citric acid–sodium citrate buffer, pH 2·6. The results of equilibrium ultracentrifugation analyses of this prekeratin strongly suggest that this protein is not homogeneous. This conclusion is in accordance with the observations of two other investigators whose data infer that there is no major homogeneous keratin precursor in cow's lip or snout epidermis.

A major advance in the isolation of prekeratin from bovine snout epidermis was accomplished by Matoltsy (1965) who extracted this tissue with 0·1 mol/l citric acid–sodium citrate (CASC) buffer, pH 2·6. This buffer extracted prekeratin from the non-keratinized layers of the epidermis after which the protein was isolated by serial precipitation. The prekeratin appeared monodisperse during ultracentrifugation and Tiselius electrophoresis, gave rise to an α-type diffraction pattern, and possessed a fibre-forming capacity (Matoltsy, 1965). Prekeratin was further characterized as having a molecular weight of 640,000, dimensions of 1050 Å by 37 Å, and an α helical content of about 40% (Matoltsy, 1965). Isolation and characterization of the α helical regions as well as the repeating subunit of prekeratin have been determined (Skerrow, 1971; Skerrow *et al.*, 1973). Evidence is presented in this report which strongly suggests that bovine snout epidermal prekeratin is not a homogeneous material.

MATERIALS AND METHODS

The cows' noses were obtained at a local abattoir. They were kept in ice and water until a sufficient number of snouts had been collected at which time they were taken to the laboratory. The fresh and ice-chilled noses were thoroughly washed with tap water, and the epidermis was removed with a Brown Electro-Dermatome. Prekeratin was then prepared according to the procedure of Matoltsy (1965). The protein content of the final preparation of prekeratin was determined by the procedure of Lowry *et al.* (1951). Sodium azide (0·02%) was added as a preservative.

Equilibrium ultracentrifugation runs were carried out on 2 samples of 0·25% prekeratin in CASC buffer and on one sample of 0·25% bovine serum albumin (BSA) in 0·05 mol/l borate buffer, pH 8·5. These samples were loaded into a 6 channel Yphantis centerpiece (Yphantis, 1964) using 3 mm columns, and were then centrifuged overnight from 18 to 26 h at the speeds indicated below in a Model E

Spinco Ultracentrifuge at 20°C. Rayleigh interference as well as Schlieren patterns were photographed at various time intervals. When it was apparent that equilibrium had been reached at one speed, a new speed was employed. Molecular weights were calculated by the method of Nazarian (1968) and as elaborated by Di Camelli *et al.* (1970) using an IBM 1130 computer. A value of 0·730 ml/g was used for the partial specific volumes of prekeratin (Matoltsy, 1965) and BSA (Kabat & Mayer, 1967).

RESULTS AND DISCUSSION

The calculated molecular weight of prekeratin depended upon the rev/min during the run (Table 1). This behaviour is best explained by molecular weight heterogeneity. The higher molecular weight components would be removed from the meniscus region by the higher centrifugal force at the higher speeds. This action leaves only the lower molecular weight components to be measured. On the other hand the average molecular weight of 69,750 for BSA was independent of the run rev/min, and this value is in close agreement with that of 69,000 recently reported for BSA (Tanford, 1968).

TABLE 1. Molecular weight of prekeratin and BSA computed by the Nazarian method from equilibrium ultracentrifugation

Experiment	Rev/min	Molecular weight	
		Prekeratin*	BSA*
1	4,908	459,000	69,000
	11,573	171,000	69,000
2	4,908	440,000	pp†
	8,225	235,000	72,000
	11,573	163,000	69,000

* Each determination is the average of two samples of prekeratin and one sample of BSA.

† Poor pattern.

Sedimentation velocity runs of 3 samples of prekeratin at different concentrations in CASC buffer were made at 59,780 rev/min at 20°C. A sedimentation rate of 4·7 S was determined by the extrapolation to zero concentration. A corrected water value of 5·5 S at 20°C was computed using the viscosity and density data for sodium citrate given by Svedberg & Pedersen (1940). This value of 5·5 S is nearly the same as that of 5·4 S reported previously for prekeratin (Matoltsy, 1965). The Schlieren patterns were very similar to those obtained by Matoltsy (1965) in that they showed a large fairly symmetrical peak with an unresolved faster component(s) which consisted of 13% of the total pattern area as estimated by planimetry.

The results indicating the heterogeneity of prekeratin in Table 1 are in agreement with those of O'Donnell (1971) who found that the keratin precursor extracted from cow's lip epidermis at low pH by formic acid was not homogeneous but was composed of a family of closely related proteins. Also Baden, Goldsmith & Fleming (1973) demonstrated that prekeratin extracted from the snout epidermis of cows at low pH with CASC buffer was not homogeneous, but consisted of several interacting polypeptide chains, one of which appeared to be non-helical in structure.

The extraction of the fibrous proteins of beef snout epidermis was first achieved by Rudall (1952) who employed 6 mol/l urea solution. This solution extracted the fibrous protein, or epidermin, and a non-fibrous or amorphous protein from the non-keratinized epidermal cells. Epidermin was reported to give an α-type X-ray diffraction pattern, the same as that of intact epidermis, and was shown by Rudall to have a low sulphur content. However, epidermin was not suitable for characterization since it had been obtained under denaturing conditions by Rudall (1952) and by Carruthers *et al.* (1955) and was shown to be heterodisperse in solution (Mercer & Oloffson, 1951; Woernley *et al.*, 1957). Thus urea solution and CASC buffer extract epidermin and prekeratin, respectively, neither of which is homogeneous. However, both of these proteins are related as shown by immunological means (Carruthers, 1973).

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Surfometry

A METHOD OF EVALUATING THE INTERNAL STRUCTURE OF THE STRATUM CORNEUM

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SUMMARY

A method is described which enables measurement to be made of the roughness of the surface of skin surface biopsies. The instrument used in this investigation has previously been employed to measure the degree of unevenness of metal and ceramic surfaces and is called a 'surfometer'. Using this technique the tracings that resulted from examination of normal skin, psoriasis and certain ichthyotic states could easily be distinguished. It was also demonstrated that there was a consistent pattern of change with increasing hydration of the stratum corneum in the absence of easily identifiable morphological alterations.

The stratum corneum is a complex heterogeneous tissue which in health sheds its component horn cells in a delicately controlled manner. This process of desquamation is of considerable importance in any consideration of epidermal homeostatis, but we are ignorant of the mechanisms involved. One reason for the lack of understanding of this important aspect of epidermal biology is that there are relatively few methods for measuring structure or function of the horny layer. In this investigation we have examined and measured the plane of dehiscence of the stratum corneum obtained by the method of skin surface biopsy (Marks & Dawber, 1971). We believe that this method of examination (which we term 'surfometry') may provide a measure of intracorneal adhesion, and in addition the findings that it yields may be useful diagnostically.

MATERIAL AND METHODS

The instrument

The instrument used in this investigation (Surfometer, Planer Products Ltd) was designed to record micron changes in the contour of metal and ceramic surfaces. It consists of a very sensitive moving stylus which transmits and records movements due to roughness in the surface over which it travels (Figs. 1 and 2). Movements are recorded on a moving chart, similar to an electrocardiograph.

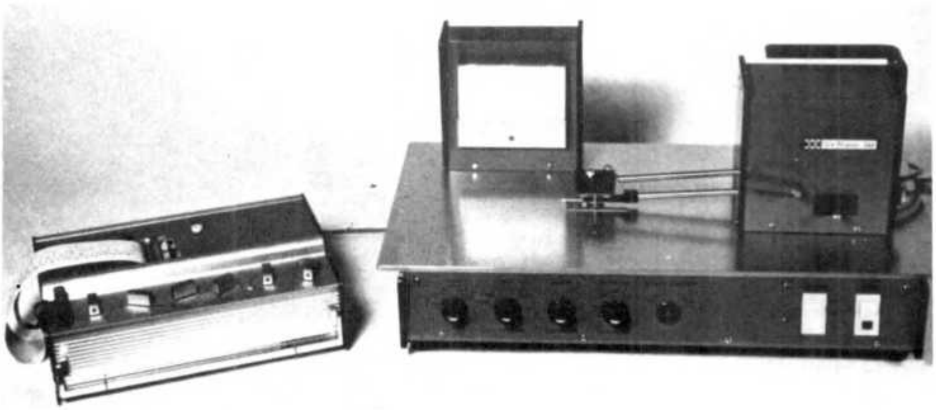

FIGURE 1. The surfometer used in this investigation.

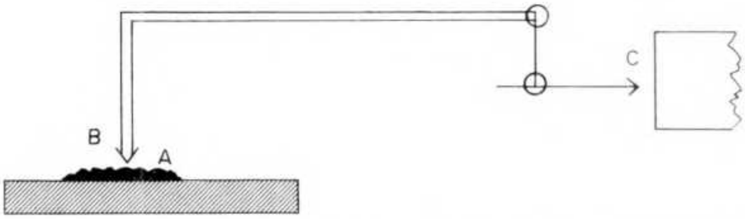

FIGURE 2. The surfometer diagramatically. A, skin surface biopsy; B, the stylus; C, the recording system.

Skin Surface biopsies (SSBs)

Skin surface biopsies (SSBs) were taken using the method of Marks & Dawber (1971). In this method a drop of cyanoacrylate adhesive (Permabond) is placed on a glass microscope slide which is then pressed against the area to be sampled. After approximately 20 s the glass slide is removed together with a layer of stratum corneum several cells thick.

The patients

(1) SSBs were taken from the forearms of eight normal individuals of mean age 29·1 years (four women; four men) and their contours recorded by the surfometer. The same site was sampled until glistening membrane was reached in three individuals and the resulting SSBs examined in the surfometer. SSBs from normal individuals were also taken from the following sites—the forehead, the upper back, the back of the hand, the lower leg and the abdomen—and examined by surfometry.

(2) In five individuals the stratum corneum of the volar aspects of the forearms was hydrated by applying water-soaked gauze swabs under polythene occlusion for different periods (1–24 h). At the end of the periods of hydration the sites were patted dry and exposed to the ambient atmosphere for 5 min. SSBs were then taken from these sites and examined by surfometry.

(3) SSBs were also taken from psoriatic lesions in six patients after the superficial scales had been gently detached, and examined by surfometry. The uninvolved skin of three patients with psoriasis was also sampled. The SSBs were examined by surfometry as before.

(4) One patient with dominant ichthyosis, one with 'xeroderma', one with non-bullous ichthyosiform erythroderma and one with lamellar ichthyosis were also investigated by SSB and subsequent surfometry.

Chart analysis

All recordings were taken with the stylus traversing the direction of the skin surface markings. The surfometer was used with the following settings; (a) chart speed of 5 mm/s, (b) a 'stylus traverse speed' of 10 mm/min, and (c) the lowest sensitivity of the surfometry recording system giving a maximum deflection of 100 µm. Representative 10 cm lengths of the tracings were used for analysis (Fig. 3).

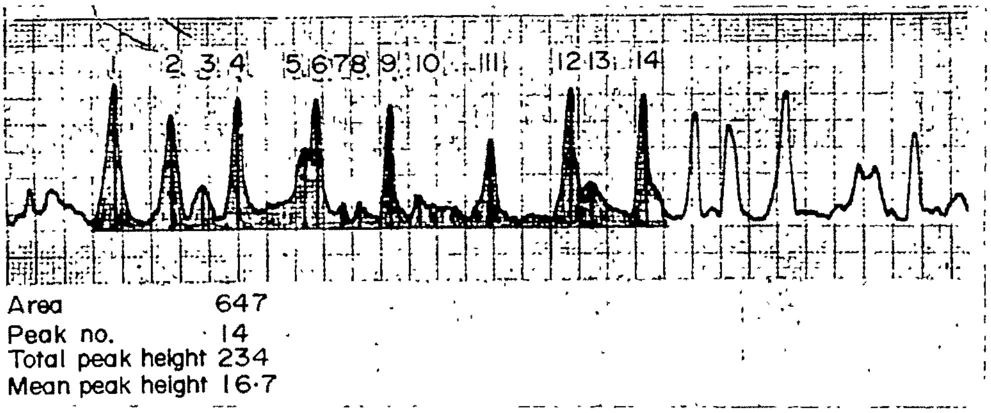

FIGURE 3. Typical normal surfometer tracing with a marked 10 cm length showing the method that has been used to quantitate representative 10 cm lengths.

Repeated 10 cm lengths were analysed in several tracings and little difference was found between them. The following values were obtained from the 10 cm lengths of chart; (1) area under the 10 cm length of tracing, (2) the number of peaks in the 10 cm length, and (3) the total height of the peaks in the 10 cm length. In order to measure the area under the tracing it was necessary to draw a line at the base which just touched the lowermost trough (see Fig. 3).

RESULTS

(1) The results of analysing surfometer tracings in eight normal adults* are seen in Table 1. There was no difference in the surfometer tracings between the sexes. There were some differences in surfometry tracings taken from different sites in the same normal individual, but these were not great and have not as yet been quantified. No age-related changes have been detected.

(2) The results of analysing the surfometer tracings taken after increasing periods of hydration are given in Table 2.

(3) SSBs were taken from six patients with plaque type psoriasis. Surfometry tracings were taken from these SSBs and analysed as described above. The results are given in Table 3. It can be seen

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* During examination of these 'normal' SSBs, repeated tracings were taken from the same area of the same specimen to determine the reproducibility of the technique. Identical tracings always resulted from this manoeuvre, indicating that the technique is reproducible and in addition that the diamond stylus does not injure or distort the surface of the SSBs.

TABLE 1. The results of analysing representative 10 cm lengths of surfometer tracings of SSBs from the forearms of eight normal adults

	Mean	s.e.m.
Area under tracing (mm²)	809·7	46·0
No. of peaks	14·5	0·5
Total peak height (mm)	267·8	15·2

TABLE 2. Analysis of surfometer tracings of SSBs taken from areas of hydrated forearm skin at increasing time intervals in five normal subjects. The surfometer tracings showed a similar pattern of changes in the five subjects at the different periods of hydration. The SSBs which were analyzed could not, however, be distinguished visually

	Subject						
Time (h)	1	2	3	4	5	Mean	s.e.m.
	Area under 10 cm tracing (mm²)						
0	873	640	1088	776	683	812·0	81
½–1	1369	743	1060	1076	839	1017·4	110
3	1525	—	959	1278	1472	1308·4	125
18–24	543	982	1229	1143	1012	981·8	120
	No. of peaks in 10 cm tracing						
0	14	19	23	18	13	17·4	1·8
½–1	9	17	19	13	16	14·8	1·8
3	18	—	19	23	12	18·0	2·3
18–24	18	16	11	16	15	15·2	1·1
	Total peak height in 10 cm tracing (mm)						
0	225	246	275	324	202	254·4	21·5
½–1	303	277	340	299	346	313·0	13·4
3	430	—	283	444	356	378·3	37·2
18–24	226	289	295	336	303	289·3	18·2

that there is a considerable difference in the 'area' measurement compared to normal. This difference is significant statistically ($P<0{\cdot}001$). The difference between the total peak heights is marginally significant ($0{\cdot}1>P>0{\cdot}05$).

The tracings from these patients with psoriasis were very irregular and 'spikey' compared to normal and seemed to have a distinctive and identifiable pattern (Fig. 4).

The uninvolved skin from two psoriatic patients was also examined. The results are given in Table 4 and do not appear to differ from the results obtained from normal specimens (Table 1).

TABLE 3. Analysis of surfometer tracings derived from lesions of six patients with psoriasis

	Mean	s.e.m.
Area under tracing (mm^2)	1551	164·8
No. of peaks	14	2·0
Total peak height (mm)	323	42·8

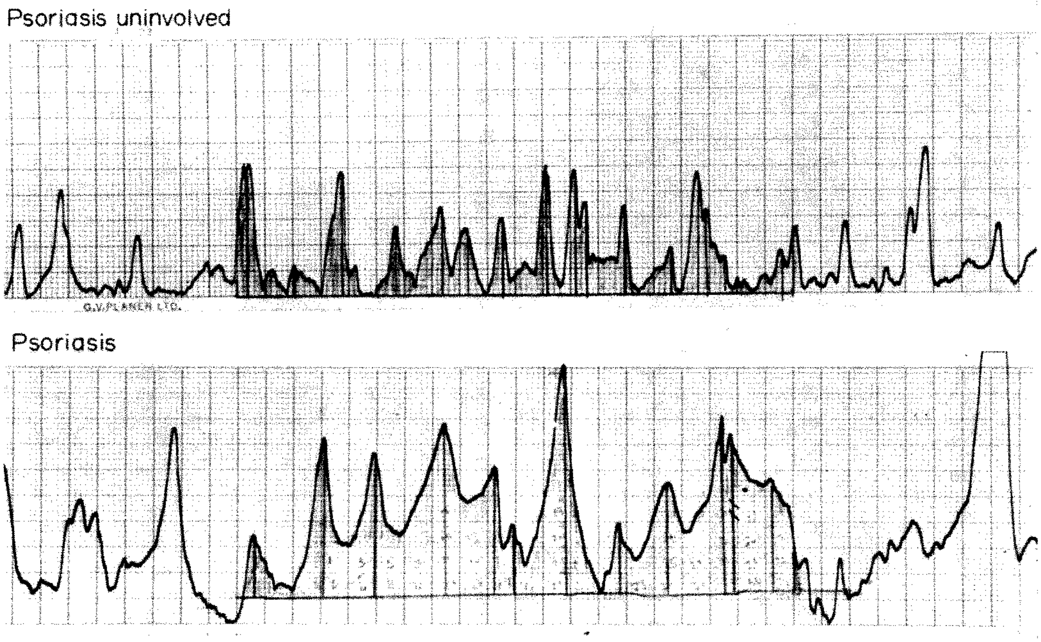

FIGURE 4. Ten centimetre lengths of surfometer tracings from a patch of psoriasis on the abdomen and from an uninvolved site on the forearm. The psoriatic tracing is easily distinguishable.

TABLE 4. Analysis of surfometer tracings from uninvolved skin of two patients with psoriasis

	Patient 1	Patient 2
Area under tracing (mm^2)	773	760
No. of peaks	17	13
Total peak height (mm)	256	236

TABLE 5. Analysis of surfometer tracings in four patients with ichthyotic conditions

	Dominant ichthyosis	Xeroderma	Ichthyosiform erythroderma	Lamellar ichthyosis
Area under tracing (mm^2)	1224	951	1234	906
No. of peaks.	5	13	13	7
Total peak height (mm)	90	198	395	107

(4) One patient with dominant ichthyosis, one with 'xeroderma' and one each with lamellar ichthyosis and ichthyosiform erythroderma were examined. The results of analysing these surfometer tracings are given in Table 5.

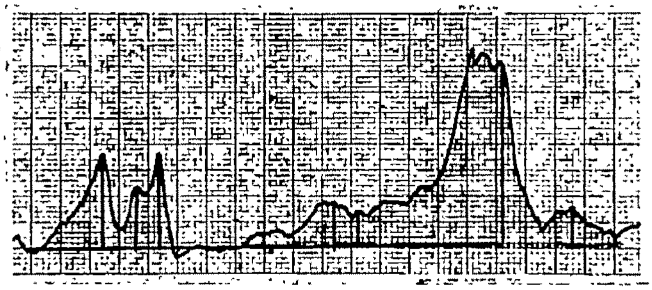

FIGURE 5. Surfometer tracing from patient with lamellar ichthyosis.

The only one of these tracings to appear distinctive was that from the patient with lamellar ichthyosis (Fig. 5).

Comment

The rate of shedding of the surface layers of the stratum corneum is ultimately dependent on the strength of the bond between individual horn cells (intracorneal adhesion). There has been no convenient way of measuring this important aspect of epidermal behaviour. Furthermore, apart from the work of McGinley, Marples & Plewig (1969) there seem to have been no other reported attempts at doing so. When an intact portion of stratum corneum is removed in the process of SSB, the 'jaggedness' of the distracted surfaces is a function of the way they were originally stuck together and the strength of the bond. A measure of the unevenness of the surface of the SSB may then reflect both the internal structure and the strength of intracorneal adhesion. The depth of the split within the horny layer must also be a function of the vertical binding forces. It is unlikely that the cyanoacrylate adhesive permeates the stratum corneum to any appreciable extent. It is a highly viscous material which polymerizes within a few seconds under pressure. Certainly the surface of the SSB seems free from adhesive both on palpation and on microscopy.

We have little doubt that the jaggedness of the surface of SSBs and the results produced by its measurement with a surfometer are due to, on the one hand, the interaction of the structure and complex internal forces of the stratum corneum and, on the other, the technique of SSB. We maintain that an important variable that determines the surface topography of the SSBs is intracorneal adhesion.

Regardless of the theoretical basis for the technique, the results presented here have an empirical interest. Psoriasis and lamellar ichthyosis are easily distinguishable from normal by the surfometry measurements. Of even greater interest is the fact that specimens of hydrated stratum corneum could be identified from non-hydrated specimens and there seems to be a consistent pattern of change with increasing hydration. There is, at the moment, a dearth of methods which can measure the degree of hydration of the stratum corneum. It may be that the simple method described here, or some development of it, will enable this situation to be remedied.

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British Journal of Dermatology (1975) **92,** 659.

Adnexal polyp of neonatal skin

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SUMMARY

The adnexal polyp of neonatal skin is a small, usually solitary tumour of the skin occurring mostly on the areola of the nipple of the neonate. Our statistics reveal an incidence of 4·0% in 3,257 newborn infants. Histologically it always contains hair follicles, eccrine glands and vestigial sebaceous glands, hence the term 'adnexal polyp of neonatal skin' is proposed. No similar report has been hitherto recorded.

The adnexal polyp of neonatal skin (APN) is an elastic, firm and polypoid tumour, about one mm in diameter or less. The surface is smooth, and the colour is normal or pink (Fig. 1). It can easily be

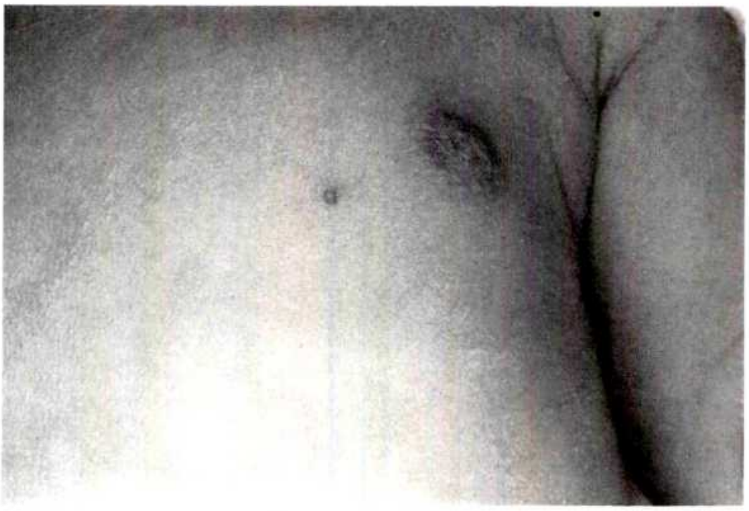

FIGURE 1. Adnexal polyp on the left breast.

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removed by the fingertip to leave minute bleeding. This tumour becomes dry and brownish and falls off spontaneously within a few days after birth.

We made a statistical observation of all newborn infants born in the Tokyo Metropolitan Police Hospital from November 1968 to October 1973, and found the APN in 129 (4·0%) of 3,257 infants. The majority were Japanese, but we found the tumour in one black infant from Zaire. While most of the affected infants had only one tumour, ten had two tumours and one had three. This brought the total to 141 tumours. In some infants the tumours were observed symmetrically on both nipples.

As shown in Table 1, the site of predilection is the areola of the nipple. Apart from one tumour on the sternal region and three perimammary tumours in which the side was not noted, seventy-eight were found on the left side and fifty-nine on the right side. Usually they were found several millimetres medial to the nipple. They seem to fall off within a week after birth, as they were not observed in infants older than one week. One 26-day-old infant was an exception.

TABLE 1. Site of the adnexal polyp of neonatal skin

	Right	Left
Lower eyelid	0	1
Cheek	1	2
Preauricular region	1	0
Scapular region	0	2
Axilla	1	0
Arm	0	1
Areola of the nipple	55	68
Hypochondrium	0	1
Scrotum	1	2
Labia majora	0	1
Total	59	78

Histology

The microscopic findings of the APN are typical. The epidermis is usually normal. At times it is covered by a thin crust, and in several tumours a small portion of the entire epidermis was necrotic and accompanied by subepidermal blisters.

The most characteristic finding is the presence of adnexal elements in the centre of the tumour. There are hair follicles, vestigial sebaceous glands and well developed eccrine glands (Fig. 2). Several follicles were curved and, at times, open to the surface. Keratinization of the hair was also noted, but no hair growing onto the skin surface. There are no epithelial strands budding from the follicles. The sebaceous gland is not always found, and when noted, it is poorly developed and attached to the follicular infundibulum (Fig. 3). There is no arrector pili. Although apocrine glands are absent, eccrine glands are usually well developed, and connection with the intraepidermal eccrine duct can be observed. The dermis contains fairly numerous small vessels, but no inflammatory infiltrate. The PAS stain and alcian blue stain are negative. The perifollicular collagen is rather loose.

All of the tumours contain adnexal structures except for those in which the epidermis seems to have dried out. These old tumours contain only one or a few keratinous cysts surrounded by a thin epithelial wall and the dermal collagen is more or less eosinophilic and dense.

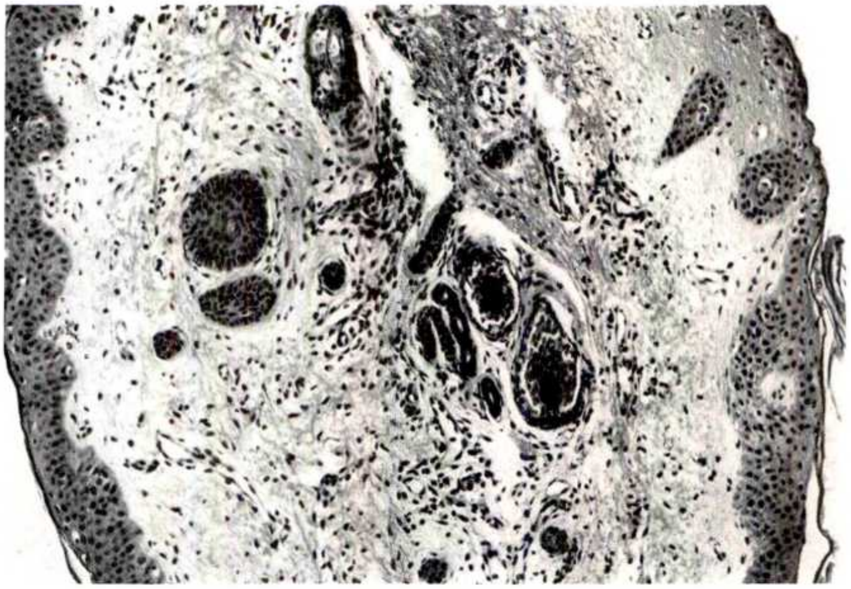

FIGURE 2. In the centre of the lesion are found several hair follicles and eccrine glands (low magnification).

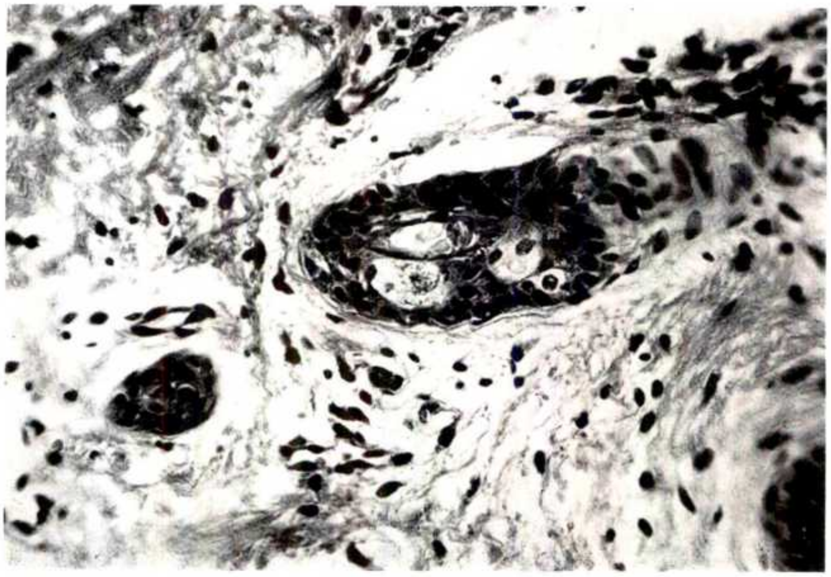

FIGURE 3. Vestigial sebaceous glands (high magnification).

Comment

The characteristics of the APN are summarized as follows:

(1) It is a small, usually solitary tumour of the skin observed exclusively in the newborn infant.

(2) The majority of the tumours are found on the areola of the nipple.

(3) Within a few days the tumour falls off spontaneously.

(4) Microscopically, hair follicles, vestigial sebaceous glands and eccrine glands are noted in the centre of the tumour.

No similar tumour has been recorded in the literature. In newborn infants, accessory auricles and supernumerary breasts are not infrequently observed, but they are different from the APN both clinically and histologically.

In the classification of tumours of hair follicle origin, Lever (1967) described the pigmentary hair naevus as an organic hamartoma, and the trichofolliculoma as an organoid hamartoma. Pinkus & Mehregan (1969) considered hair naevus, hair follicle naevus and trichofolliculoma as the best organized tumour of the pilosebaceous complex. For Duperrat & Mascaro (1965), naevus pilaire, trichofolliculoma and dermatosis papulosa nigra are 'hamartome parfait' of follicular origin. Apart from the presence of eccrine glands and the lack of epidermal hyperplasia, the APN is histologically quite similar to dermatosis papulosa nigra which contains immature hair follicles showing little tendency to sebaceous differentiation and occasional keratinous cysts.

The degree of differentiation of the APN is about the same as organoid hamartomata. However in neither tumour nor naevus, as cited above, has the coexistence of eccrine glands been reported. On the other hand, in the eccrine naevus there is no pilosebaceous apparatus mixed in the tumour. Naevus syringosebaceus and naevus syringopilosebaceus in Nikolowski's paper (1958) show the coexistence of apocrine and sebaceous or pilosebaceous hamartomata, but in these tumours the apocrine and pilosebaceous elements grow side by side, not mixing as in the APN. Therefore, the APN is considered a unique hamartoma of syringo-pilo-sebaceous origin.

The reason why the tumour falls off spontaneously remains unsolved. In tumours obtained from 4- or 5-day-old infants, the epidermis seems to be dried, but neither necrosis nor thrombosis could be observed in the corium. The adnexal components have regressed and only keratinous cysts are occasionally found in the centre of the tumour.

We hesitate to call a naevus or hamartoma a tumour when it falls off spontaneously within a few days of life. For this reason, the term adnexal polyp of neonatal skin is proposed.

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Mucocutaneous leishmaniasis in guinea-pigs inoculated intravenously with *Leishmania enriettii*

PRELIMINARY REPORT

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SUMMARY

Seven guinea-pigs were inoculated intravenously with a rich suspension of *Leishmania enriettii*. During the sixth to the seventh week from inoculation, all of them developed lesions within the anterior nasal mucosa and in the vulva or scrotum. In one animal nodules occurred in the forepaws during the tenth week. The histology of the latter showed an epithelioid granuloma with multinucleate giant cells, while in all the others macrophages and monocytes highly parasitized with leishmania bodies, formed the main elements in the granulomatous infiltrate. Lesions seem to develop in the cool, non-hairy areas of the integument and those areas of the nasal mucosa which are constantly bombarded by the inhaled cool air stream. The anterior nasal mucosa, with its leaking venular network, its cool temperature, relative stasis and hypoxia probably provides the optimum micro-environment for the localization, survival and multiplication of the leishmanial protozoon.

Spontaneous guinea-pig leishmaniasis was first described and its causative protozoon, *Leishmania enriettii*, was identified by Medina in 1946 in South Brazil. Later, Muniz & Medina (1948) were able to reproduce the disease in a batch of twenty-five guinea-pigs inoculated subcutaneously with *L. enriettii*. They could not, however, produce visceral or mucous membrane lesions. During the course of the disease process they observed new lesions in the skin, in regions situated far from the site of inoculation such as the forepaws, ears, hind paws and genital organs. In their opinion such lesions occurred as a result of auto-inoculation of the non-hairy areas through scratching and contamination of the animal's nails from the primary site.

In spite of many attempts they also failed to reproduce the disease in different mammals including the rhesus monkey, hamster, dog, prea, white rat and mouse by using very rich suspensions of infected tissue as well as of culture forms of *L. enriettii* either subcutaneously or through the peritoneum. The following is a preliminary report of findings in seven guinea-pigs inoculated intravenously with a rich suspension of tissue form of *L. enriettii*.

MATERIALS AND METHODS

Preparation of the inoculum

The strain of *L. enriettii* used in this experiment has been maintained through passage into successive batches of guinea-pigs by subcutaneous inoculation by the kind care of Dr David Bradley of the Radcliffe Infirmary, Oxford. A freshly excised nodule from the cranial skin of the guinea-pig ear inoculated 2 months previously was dissected and the granuloma neatly obtained and put in a sterile Petri dish. It was sliced into small fragments which were transferred into a manual homogenizer where it was crushed with the glass mortar handle through slow, pressing rotary movements. The supernatant in the resulting homogenate was then sucked off with a Pasteur pipette and centrifuged at 2000 rev/min for 10 min. The supernatant was discarded after checking a drop of it for absence of leishman bodies with the phase contrast microscope. After adding some of the special fluid medium to the precipitate at the bottom of the centrifuge tube, the resulting suspension was mixed by repeated aspiration using a Pasteur pipette. The same tube was then transferred to an electric vibrator for further homogenization of the suspension. Using a counting chamber the selected dose could be calculated accordingly.

The animals and dose of inoculum

Seven guinea-pigs, four males (average weight 420 g) and three females (average weight 360 g), were used. Under open ether anaesthesia the femoral vein was exposed in the ventral aspect of the thigh. In a group of four animals of both sexes an inoculum of 10 million *Leishmania* organisms per animal was administered into the femoral vein using a No. 25 hypodermic needle. In another group of three animals a dose of 5 million *Leishmania* organisms per animal was similarly given. After suturing the wound and marking each animal by punching the ear lobe, each animal was kept in a separate cage in the animal house for further follow-up. The animals were examined twice weekly for any lesions and both colour and black and white photographs were taken whenever a lesion appeared or a change in the clinical picture occurred.

FOLLOW-UP AND RESULTS

Macroscopical findings

After a period of 5–6 weeks from the day of inoculation each of the seven animals developed a clinically apparent endonasal nodule in the tip of the maxilloturbinal or the nasoturbinal. In some of the animals the lesion appeared within the right nostril (Fig. 1) while in the others it occurred in the left nostril (Fig. 2). In two animals it occurred in the anterior swell of the nasal septum. Almost simultaneously a nodular swelling was observed to occupy one side or the upper part of the scrotum or vulva (Figs. 1 and 2). In one animal only, nodules occurred in both forepaws during the tenth week after inoculation. Further follow-up of the lesions showed local extension and ulceration with secondary infection probably through self scratching.

Later two of the animals were found dead in their cage at the end of 10 weeks from the day of inoculation. A post-mortem on each of them revealed a large ischiorectal abscess spreading from the vicinity of the leishmanial lesion in the external genitalia. The internal organs showed signs of septicaemia with bilateral patchy pneumonia. In view of the severe soreness and inflammation of the lesions at the mucocutaneous junctions of the nasolabial and anogenital sites it was decided to sacrifice the rest of the animals.

Histological findings

Sections prepared from the nasal, vulval, scrotal and paw lesions were stained with H & E and Giemsa stains. Imprints as well as paraffin sections were also prepared from slices of the liver, spleen, bone marrow, regional lymph nodes and lungs of four of the animals. No leishmanial bodies or lesions were

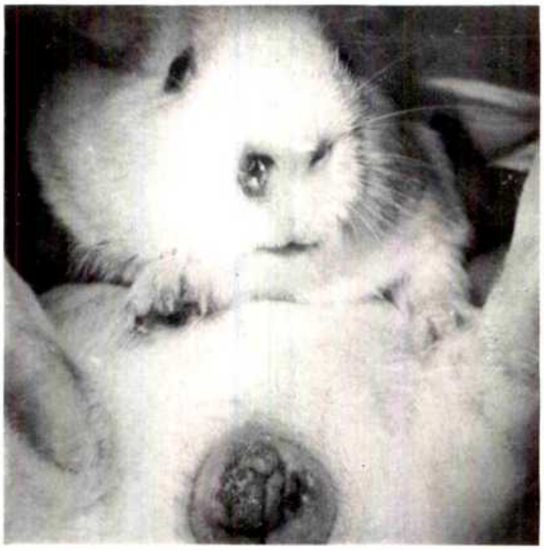


FIGURE 1. Eroded leishmanial lesion arising within the right nostril from the tip of the right nasoturbinal of a few days duration in male guinea-pig No. 1. Another lesion is seen in the upper half of scrotum.

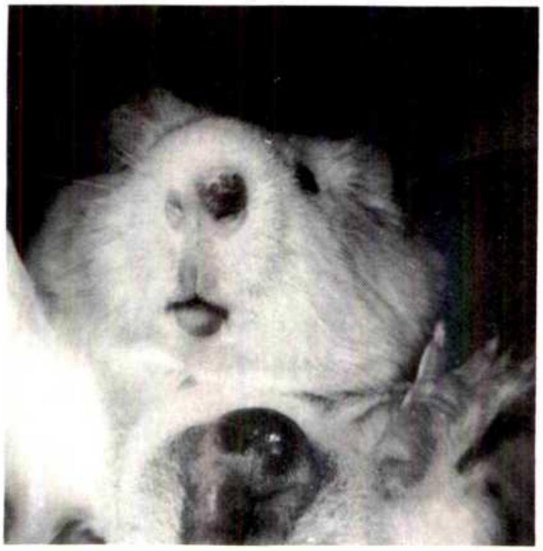


FIGURE 2. Leishmanial nodule arising within the left nostril from the tip of the maxilloturbinal and involving the dorsal part of the anterior swell of the nasal septum. Another leishmanial lesion is seen in the upper and right area of the scrotum in male guinea-pig No. 2.

found in any of the internal organs or regional lymph nodes. Sections from the endonasal nodules in all the animals revealed infiltration of the lamina propria of the tips of the involved maxilloturbinals and nasoturbinals, with highly parasitized macrophages and monocytes (Fig. 3). Clusters of roundish

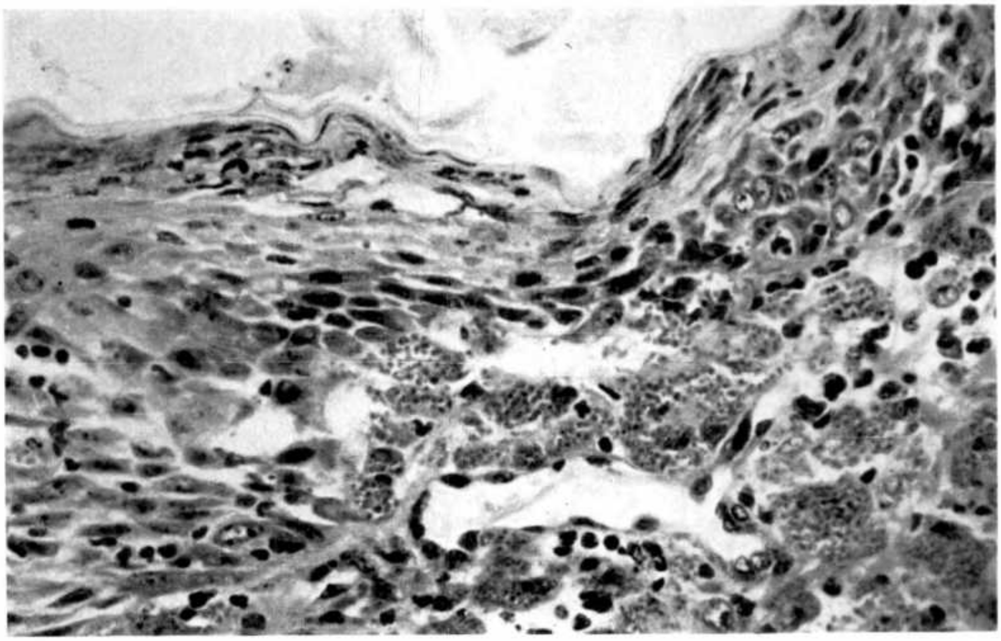


FIGURE 3. Anterior mucosa of nasoturbinal with monocytes and histiocytes highly parasitized with *L. enriettii* in lamina propria. A few mononuclear cells and polymorphs are intermingled (H & E, ×250).

or oval bodies 3–4 μm in diameter of *L. enriettii* were seen mainly in host cells in close vicinity to venules and venular capillaries in the nasal mucosa. Some of these bodies were seen even in the covering epithelium either in intercellular vesicles or in the prickle cells with a few degenerate protozoa extruded in the corneal crust on the surface of the lesion. Among the parasitized histiocytes some plasma cells and a few lymphocytes were found together with several polymorphs in the superficial part of the lesion.

Sections from the scrotal and vulval lesions revealed a similar granulomatous infiltrate which occupied the whole depth of the dermis with clear evidence of intra-epithelial phagocytosis of the parasite in the covering epidermis (Fig. 4). Sections from the paw lesion showed a different histological picture. The granulomatous infiltrate was of a more mixed pattern with a smaller number of parasitized histiocytes and monocytes in one part of the lesion but more epithelioid cells and several multinucleated giant cells in the other part of the lesion intermingled with plasma cells, lymphocytes and occasional eosinophils (Fig. 5).

Ultrastructural findings

Cubes of 1 mm were obtained from the centre of a few nodules excised from the nose, scrotum and vulva. These were immediately fixed in 3% glutaraldehyde in 5% sucrose phosphate buffer + 1% $CaCl_2$ for 3 h on ice, washed with the same buffer and post-fixed in 1% osmium tetroxide diluted in the buffer for 2 h at room temperature. Then the specimens were dehydrated in graded alcohols, treated with epoxypropane and embedded in Taab araldite. Sections were cut on IKB III ultramicrotome and viewed in Siemen's 101 electron microscope. 1 μm thick sections were also prepared and stained with toluidine blue and examined by the light microscope.

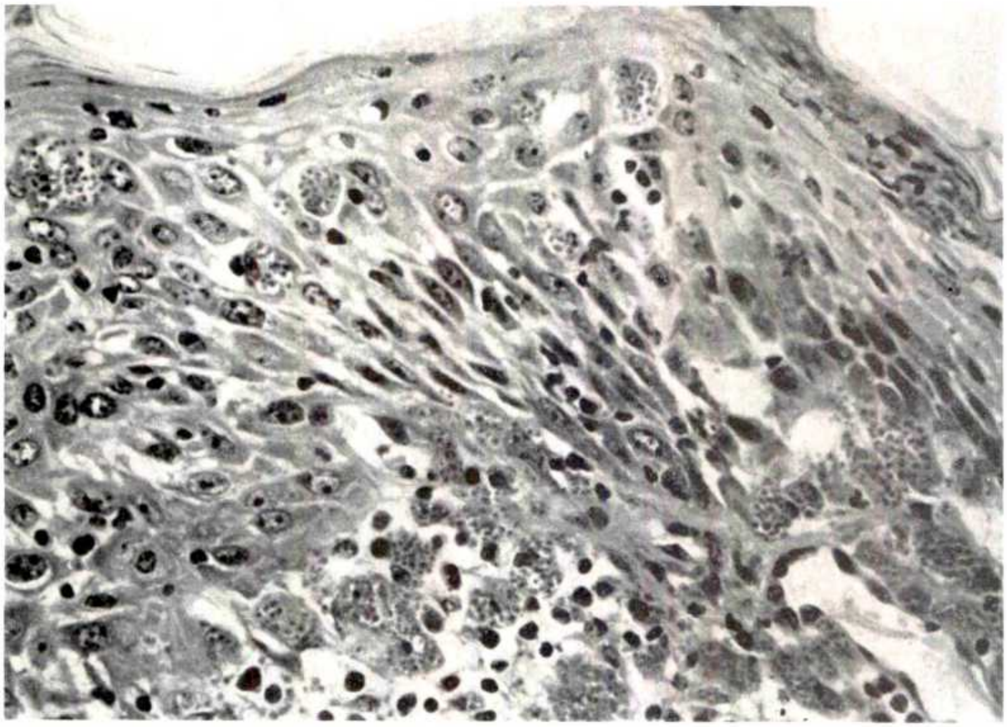


FIGURE 4. Skin of vulva infiltrated by a large number of parasitized monocytes and histiocytes mainly in the dermis. Phagocytosis of leishmanial protozoa by the prickle cells of the epidermis is evident even at this magnification (H & E, × 250).

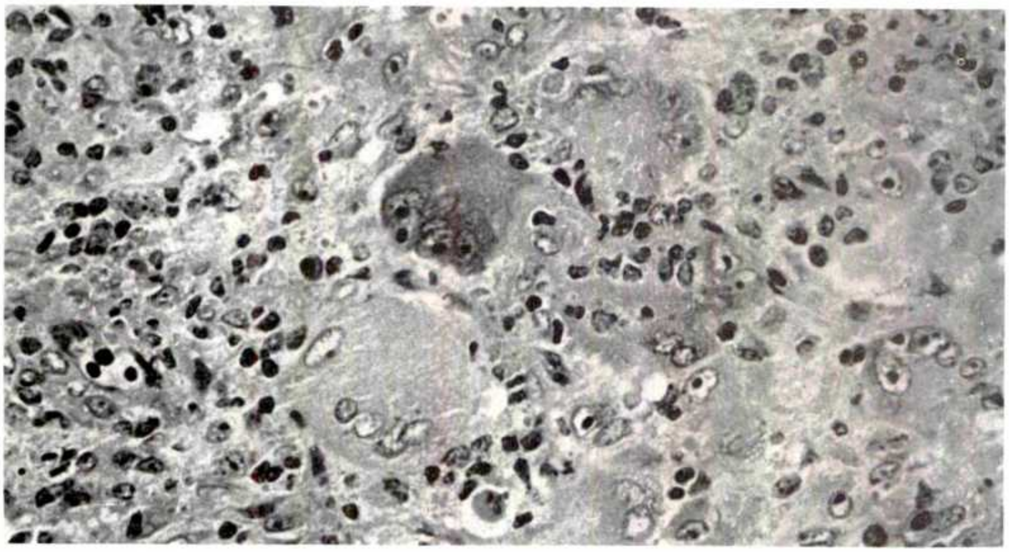


FIGURE 5. Part of forepaw lesion showing leishmania-free epithelioid cells and multinucleated giant cells mixed with lymphocytes, plasma cells, a few mast cells and eosinophils (H & E, × 250).

Numerous protozoal bodies occurred mainly in histiocytes in the lamina propria of the anterior nasal mucosa of the turbinates and in the dermis of the scrotal and vulval skin. Groups of two, three or more leishmanial bodies were interiorized in one or more phagolysosomes of host macrophages (Figs. 6 & 7). Some of these organisms showed signs of degeneration (Fig. 7). Aggregates of host cell mitochondria and a few lysosomes were frequently found in close vicinity to the phagocytosed organisms while most of the parasitized histiocytes occurred in the vicinity of venular vessels. However, no organisms could be encountered inside polymorphs, eosinophils, lymphocytes or plasma cells or in the lumina of blood vessels in the sections studied. One of the leishmanial bodies was observed

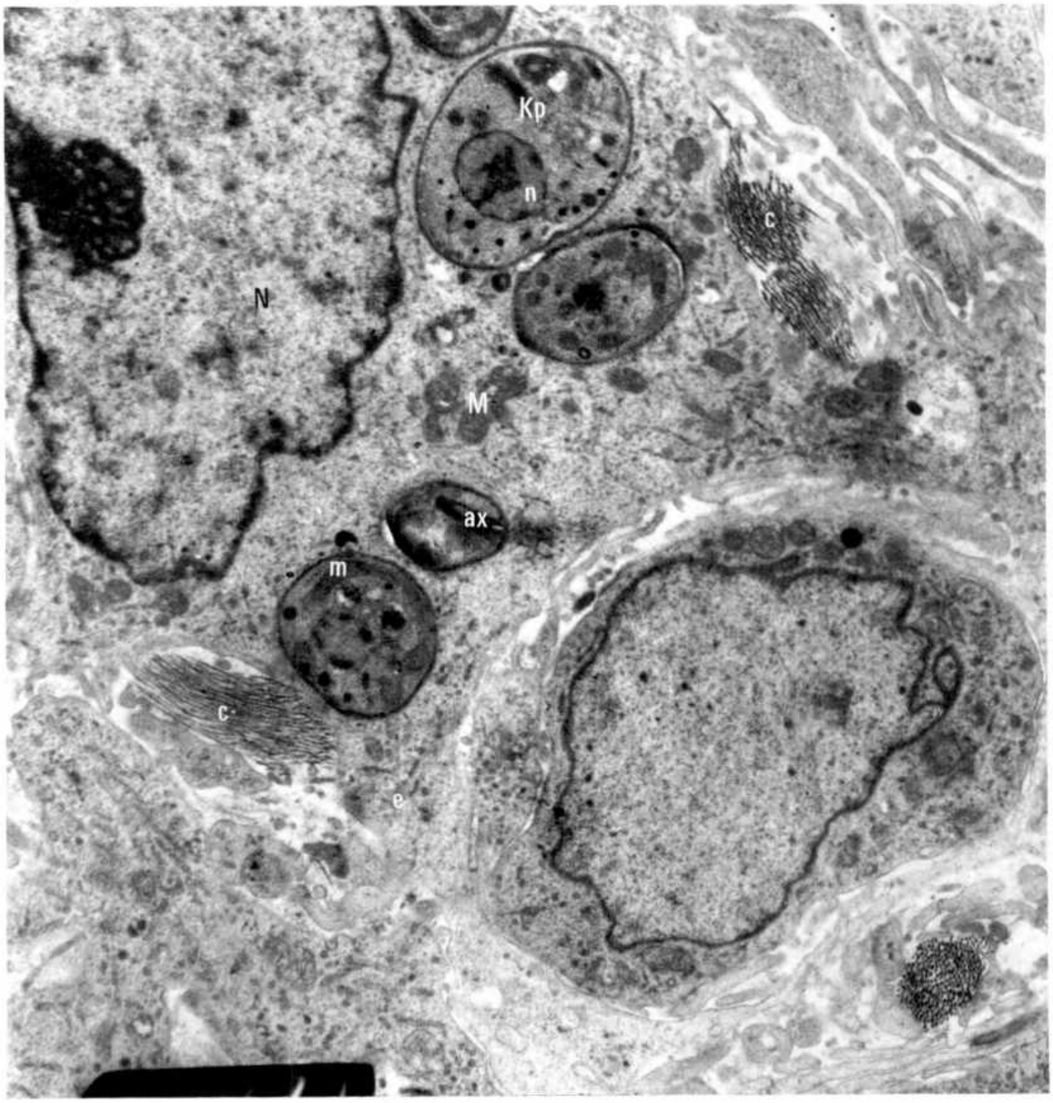
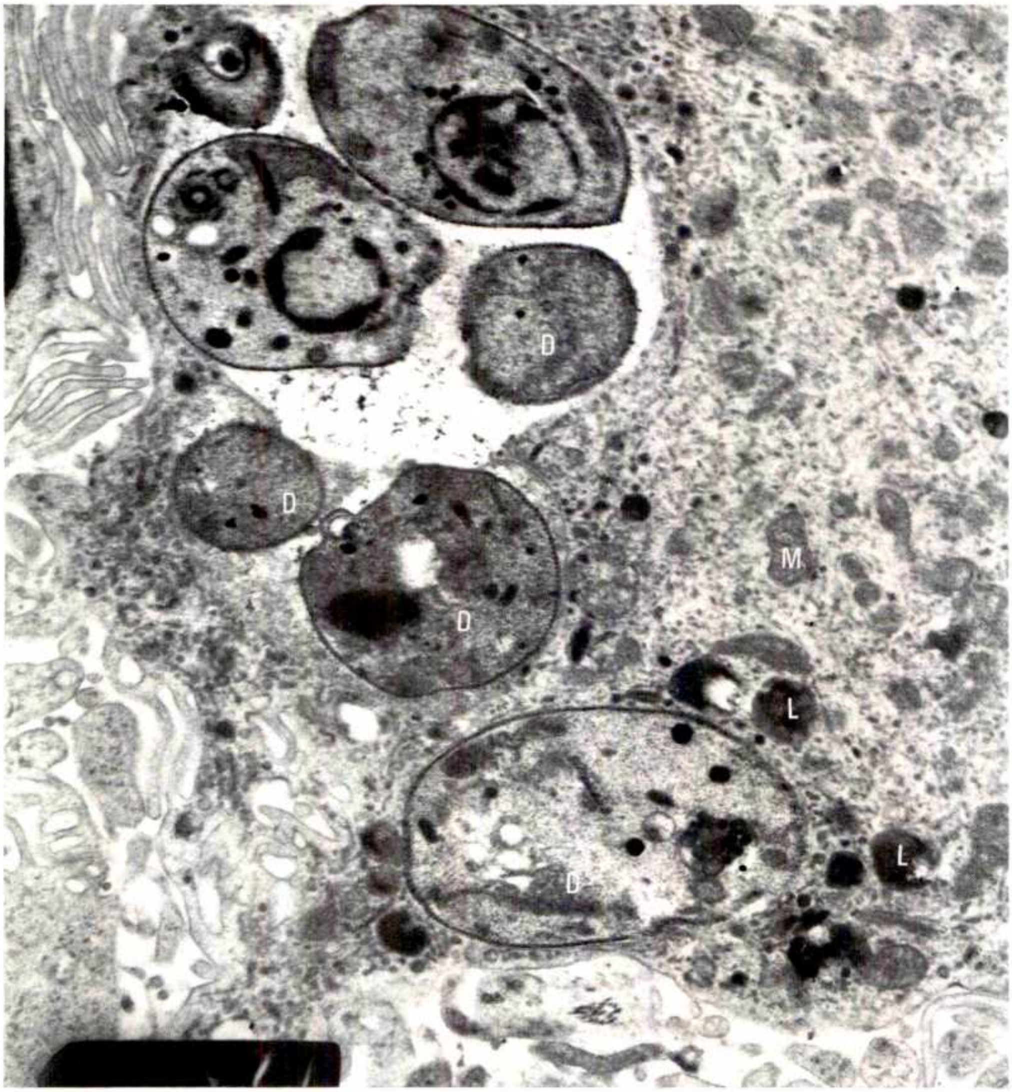


FIGURE 6. Electron micrograph showing part of histiocyte containing five leishmanial protozoa. c, collagen fibres in connective tissue; N, nucleus of histiocyte; n, nucleus of *L. enriettii*; Kp, kinetoplast; M, mitochondria of host cell; m, protozoal mitochondrium; ax, axoneme of leishmania (× 7700).



FIGURES 7. Leishmanial bodies interiorized within intracytoplasmic phagolysosomes of host cell. D, degenerate leishmanial bodies with broken periblasts and debris of their organelles. A few lysosomes (L) and many mitochondria (M) of the host cell are seen in the vicinity (× 13,4000).

during the process of phagocytosis being engulfed between two protoplasmic processes of a monocyte while the latter had already been interiorized into another macrophage (Fig. 8).

Like other flagellates of the genus *Leishmania* the fine structure of *L. enriettii* showed a nucleus with one or two karyosomes, a kinetoplast contained in a huge mitochondrium, and a flagellar structure with a single axoneme (rhizoplast). Details of this are to be published elsewhere (Kanan, 1974).

DISCUSSION

Unfortunately the follow-up in this experiment was short because of the superimposed bacterial

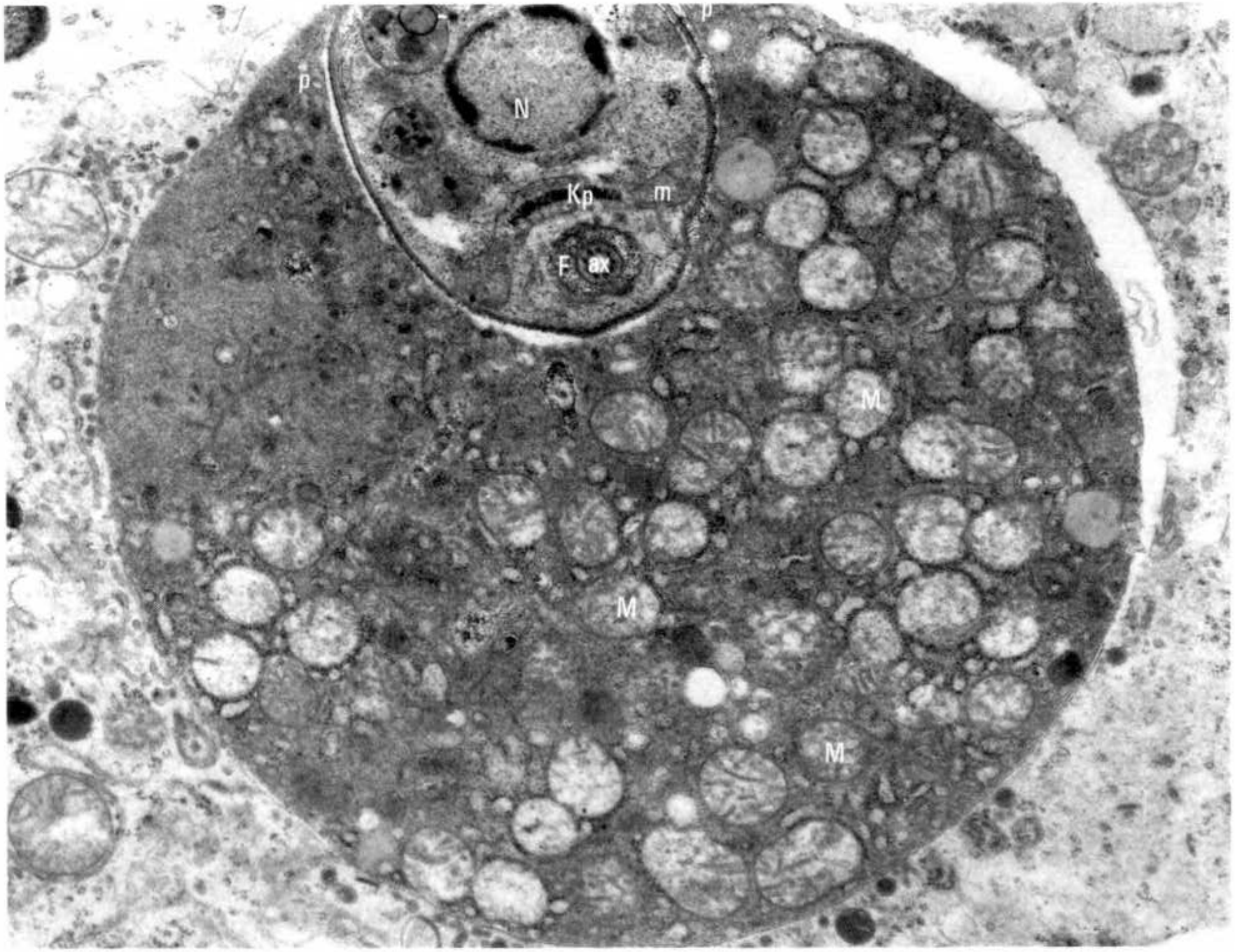


FIGURE 8. A monocyte engulfing a leishmanial protozoon is seen surrounded by a histiocyte. M, mitochrondria of monocyte; P, protoplasmic processes engulfing the protozoon; N, protozoal nucleus; m, protozoal mitochondrium; Kp, kinetoplast; F flagellar pouch; ax, axoneme in cross-section with its nine pairs of hollow fibrils (× 17,100).

infection which ended in frank septicaemia, pneumonia and death in two animals while the rest of the animals had to be sacrificed because of severe soreness and ulceration at the site of the mucocutaneous lesions. Nevertheless the successful production of mucocutaneous leishmanial lesions in the endonasal mucosa and external genitalia in every inoculated guinea-pig in this series supports the view that the metastatic lesions away from the inoculation site in subcutaneously infected animals could be due to haematogenous spread. This is contrary to Muniz and Medina's conception (1948) that cutaneous lesions remote from the site of inoculation were always caused by auto-inoculation. Their view was based on the absence of mucosal or visceral lesions in the series of guinea-pigs which they had inoculated subcutaneously.

Paraense (1952), however, succeeded in producing endonasal lesions mainly in the turbinates in eight out of ten guinea-pigs which he had inoculated intraperitoneally with *L. enriettii*. His findings (1952, 1953) strongly support a blood borne mode of infection for the metastatic lesions whether they were cutaneous, endo-osseous or mucosal.

The results of the present experiment again add further support to Kanan & Ryan's opinion (1975), that irrespective of the portal of entry the causative organism of most of the granulomatous diseases in the nasal mucosa develop there as a result of haematogenous spread. It may be relevant to refer to Brown & Pearce's experience (1920) with experimental syphilis in the rabbit. In like manner they found that lesions developed with considerable frequency in parts of the body where skin and mucous membranes join. They believed that the transitional area exercises some influence upon the localization of the infection. Majno (1965) expressed the opinion that the preference of certain diseases for specific sites may partly depend on the local vascular pattern, particularly on the distribution of the venules, the latter being more susceptible to injury of many kinds. Dawes & Prichard (1953) in a comprehensive study of the nasal angio-architecture in the dog, cat, rabbit, rat, goat, sheep and pig showed that the most striking feature of the venous system within the nose was the great size and number of the veins compared with those of the neighbouring arteries. These vessels as well as the glands were most numerous beneath the surface of the mucosa of the maxilloturbinals and the nasoturbinals which are most exposed to the inspiratory air stream. Kanan (to be published) has shown that intravenously administered colloidal particles are entrapped by the endothelium of the capillaries and venules, the pericytes and the macrophages in the lamina propria of the rat, mouse, guinea-pig and goat mainly in the anterior parts of the nasal septum, middle and inferior turbinates. The author has also demonstrated that the trapping of such particles occurs under the side of the mucosa which faces the inhaled air stream. In another experiment (Kanan, in preparation) this phenomenon was enhanced by exposure of the animal to the cold and was also influenced by certain hormones. Moreover, the histopathological findings in the present experiment indicate that the sites of predilective localization in the experimental guinea-pig, especially the nasal lamina propria, provide a good culture medium for *L. enriettii*. Similar findings have been reported in a recent histopathological study of early lepromatous leprosy in the human nose (McDougall *et al.*, 1975), and in the nose of the experimental mouse (Rees, 1966; Rees, McDougall & Weddell, 1974).

In guinea-pig leishmaniasis metastatic granulomatous lesions seem to develop in the cooler areas of the body, especially those uncovered by the hairy coat. This phenomenon has also been confirmed in a series of experiments on *L. brasiliensis* in the hamster by Zeledon, DeMonge & Blanco (1965). In the experience of many workers most of the granuloma-producing organisms seem to survive and multiply in tissues where the temperature is cooler than the normal body temperature (reviewed by Kanan and Ryan, 1975). The nasal mucosa lining the anterior parts of the septum, middle and inferior turbinates loses a lot of its heat to the inhaled air stream during each inspiration, hence the constant bombardment of this area by the cool air front is probably the major factor that determines the peculiar pattern of the angio-architecture described by Dawes & Prichard

(1953). Among others, local oxygen tension has been emphasized by several workers as a decisive factor in tissue resistance to a particular micro-organism (Chandler, Allison & Gerszten, 1965; Novy & Soule, 1925; Lurie, 1963).

In a recent investigation Kanan & Silver (in preparation) using microprobes for measuring tissue oxygen tension, found an area of relative hypoxia in the anterior mucosa of the nasal septum and maxillo- and nasoturbinals in the goat a few microns deep to the surface epithelium. A figure of 65–70 oxygen tension was obtained in this area while higher values of 90–95 were recorded at a corresponding level in the septum and turbinates more posteriorly. It is too early to evaluate the significance of this finding as a factor in the survival and pathogenicity of micro-organisms such as *lepra* bacilli, *leishmania*, *treponema* and some fungi which frequently localize and cause mutilating granulomatous diseases in this part of the nose (Kanan & Ryan, 1975). An interesting aspect of this study was the observation of the late developing nodules in the forepaws of one of the animals. The histopathological picture of a mixed epithelioid granuloma with several non-parasitized epithelioid and multinucleated giant cells and some parasitized macrophages with other inflammatory cells is reminiscent of some border-line lesions in leprosy.

A moderate degree of late developing cell mediated immunity might have fortified some of the parasitized histiocytes thus transforming them into parasite-free epithelioid cells and multinucleated giant cells. On the other hand the histopathological picture of highly parasitized macrophages in the endonasal and genital lesions is reminiscent of a similar picture in lepromatous leprosy. This is probably connected with the cool temperature in these sites being conducive to the survival and multiplication of such micro-organisms (Binford, 1956; Brand, 1959; Shepard, 1965; Shepard & Habas, 1967). According to Eggston & Wolff, (1947) the temperature in the nasal mucous membrane is only 30·4–32°C. Purtilo *et al.*, (1974) demonstrated a depression of transformation of lymphocytes *in vitro* both at 33 and 28°C. This phenomenon occurred using both human and armadillo lymphocytes which led them to the conclusion that cool temperatures probably depress cell mediated immunity *in vivo*. Such a depression of cell mediated immunity could explain the susceptibility of armadillos to infection with *M. leprae*. Similarly the high parasitic index in guinea-pig leishmaniasis in the cool areas of the body, including the anterior nasal mucosa, could be explained on the same basis.

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Skin manifestations associated with tumours of the brain

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SUMMARY

In a 4-year period seventy-seven patients with tumours of the brain were investigated for skin manifestations. These were observed in twenty-four (31·2%) and fall into three main groups:

Pruritus. Thirteen patients complained of pruritus. Most characteristic is the pruritus of the nostrils, which was observed in six patients. It can be extremely severe and is a sign of an advanced tumour.

Pigmentary disorders were observed in seven patients. In two, vitiligo appeared simultaneously with the brain tumour symptoms. In the others, hyperpigmentation appeared on the face later in the course of the disease.

Hyperkeratotic changes were manifest in four patients. Two had a mild hyperkeratosis of the palms and soles. The other two developed in addition an ichthyosiform atrophy of the skin.

The possible pathogenesis of the skin manifestations associated with brain tumours is discussed.

Paraneoplastic skin symptoms are of particular interest for their diagnostic and prognostic value as well as for the light they throw on the relationship between the neoplasm and the host. In spite of this interest, the skin manifestations associated with tumours of the brain have been grossly overlooked. With the exception of a few scattered reports, in which pigmentary disorders, vitiligo and pruritus are mentioned as accompanying symptoms (Andersen & Wernoe, 1930; Andreev, 1964; Andreev & Petkov, 1971; Bersaques & Hoffmann, 1971; Forman, 1952; Haxthausen, 1932; Kapp *et al.*, 1967; Lassus *et al.*, 1972), there is not one comprehensive and reliable clinical and statistical study of the problem.

In the course of 4 years (1970–1973) we examined for skin changes all patients with brain tumours treated at the Neuro-Surgical Department, Academy of Medicine, Sofia. We found that skin symptoms were considerably more common than expected and that they could be of diagnostic and prognostic interest for the neuro-surgeon.

MATERIALS AND METHODS

Seventy-seven patients with different tumours of the brain were thoroughly examined for skin changes and were divided into two groups.

Group I. Skin manifestations which, with a reasonable degree of certainty, might be considered as caused by the tumour; they include symptoms which appeared at the same time as the symptoms of raised intracranial pressure and were favourably influenced by treatment of the brain neoplasm.

Group II. Skin manifestations that were probably caused by the cerebral tumour; they include symptoms which appeared simultaneously with those from the brain tumour. However, because of the inoperability of the latter, they could not be proved as tumour dependent.

For the purpose of this study the term tumour of the brain includes tumours of the meninges (meningioma) intracerebral tumours (astrocytoma, glioblastoma, oligodendroglioma, medulloblastoma), and metastatic tumours to the brain. Patients with tumours of the hypophysis and with congenital tumours (craniopharyngioma, dermoid cysts) are not included because the skin changes with these particular tumours are well known, nor are patients with tumours of vascular origin, which are not neoplastic.

RESULTS

Table 1 shows the different types of brain tumour and the number of patients with skin manifestations. Skin changes were not associated with any special type.

TABLE 1

	No. of cases	No. with skin changes
Meningioma	21	6
Glioblastoma	20	7
Astrocytoma	11	4
Medulloblastoma	8	2
Oligodendroglioma	6	1
Metastatic tumours	3	1
Other intracerebral tumours	8	3

Table 2 shows the relationship between the age of the patients with brain tumours and the number with skin manifestations.

TABLE 2

Age (years)	No. of cases	No. with skin changes
0–10	2	1
11–20	3	2
21–30	12	3
31–40	18	6
41–50	22	6
51–60	13	4
61–70	6	1
71–80	1	1

With $P > 0{\cdot}02$ this relationship is also not statistically significant.

Skin manifestations that could be attributed to the underlying malignancy were observed in twenty-four patients (31·2%). They fell into 3 main groups, pruritus, pigmentary disorders and hyperkeratotic changes.

Pruritus. Thirteen patients complained or gave evidence of pruritus. In seven of them the pruritus was generalized and in the remaining six was localized to the nostrils. In two patients generalized itching subsided after successful removal of the brain tumour.

Most characteristic was the pruritus of the nostrils which was ferocious and persistent so that patients could be seen scratching for hours. Even when unconcious they scratched their nostrils and if restrained tried to rub their noses on the pillow. This particular kind of pruritus was never observed with other malignant diseases, nor with non-malignant disorders of the central nervous system. It might be accepted as pathognomonic for advanced brain tumours.

Generalized pruritus in neoplastic diseases has been considered as an allergic reaction to tumour specific antigens. The pruritus of the nostrils however is obviously of a different pathogenesis. It was observed only in patients with advanced tumours, infiltrating the base of the fourth ventricle.

Pigmentary Disorders. These were observed in seven patients. In two vitiligo appeared simultaneously with the brain tumour symptoms. The surgical removal of the neoplasm did not bring immediate improvement in the skin condition but one year later the vitiliginous plaques were far less numerous.

In the remaining five patients hyperpigmentation of the chloasma type appeared on the face. In one patient the clinical manifestations could be identified as 'linea fusca' or 'brown forehead ring' of Anderson & Wernoe (1930). In three patients the hyperpigmentation resolved after surgery.

Hyperpigmentation is not rare in diseases of the central nervous system and has been observed with encephalitis and with Parkinson's disease (Andersen & Wernoe, 1930; Haxthausen, 1932; Michel, 1958; Robertson, 1933). There are several theories (all of them more or less speculative) trying to explain this phenomenon, for example: stimulation of the transformation of tyrosine intermediate substances into melanin in the extracellular space; or stimulation of tyrosinase activity. The invasion of the hypophysis by the malignant growth might cause pigmentary disorders through disordered production of MSH. According to Haxthausen (1932) the pigmentary changes are caused by the involvement of strictly localized areas in the mesencephalon and in the vegetative centres. Simon (1936) however believed that they are not specific for brain lesions (encephalitis) but are caused by 'neurovegetative' and 'endocrine-sympathetic' influences, originating in a 'neuro-vegetative dystonia'.

Vitiligo in patients with brain tumours has been observed by Lassus *et al.* (1972). The theory that it is of autoimmune origin is corroborated by the observation of vitiligo in malignant melanoma (Balabanov *et al.*, 1969). Antimelanin antibodies have been identified in the serum of such patients. On the other hand, clinical observations of Lerner *et al.* (1966) suggest that the central nervous system plays some role in vitiligo.

Hyperkeratotic Changes. These were manifest in four patients. Two of them had a mild hyperkeratosis of the palms and soles and two developed in addition an ichthyosiform atrophy of the skin. In the latter two patients the skin cleared completely after the brain operation. The vitamin A and carotene levels in the sera of all patients were normal.

Palmo-plantar hyperkeratosis in patients with visceral carcinoma was reported by Neuber (1930), Dobson *et al.* (1965) and Howel-Evans *et al.* (1958). All patients suffered from neoplasms of the gastrointestinal tract. We report the association for the first time with tumours of different localization.

CONCLUSION

This study suggests that skin manifestations with brain tumours are far more common than was suspected. Their neglect is due largely to the underestimation by the brain surgeon of their value as diagnostic and prognostic signs. Even such an obviously pathognomonic symptom as the pruritus of the nostrils is not mentioned in comprehensive textbooks of brain tumours.

There is no doubt that the skin manifestations are of very limited diagnostic value. Pruritus, pigmentary and hyperkeratotic disorders are so common in dermatological practice that they should be evaluated only in connection with other, more specific symptoms. The pruritus of the nostrils is an exception as it is absolutely specific. However it is a symptom of advanced brain tumours and consequently without significance in the early diagnosis of brain neoplasms.

Statistical analysis does not reveal a significant relationship between the histology of the tumour and the skin symptoms. In the absence of a comparable study, we feel that the localization of the tumour is of greater importance for the skin symptoms than is the histological structure.

It seems significant that only three groups of paraneoplastic skin manifestations were recorded in this series. Such recognized paraneoplastic dermatoses as the figurate erythemas, acanthosis nigricans, dermatomyositis, bullous (pemphigoid) eruptions or herpes zoster were not observed. The restricted number of observations may explain this. However, the functional disorders caused by organic brain lesions may have a more specific character. Only the observation of a larger number of patients from several neuro-surgical centres can lead to a definite answer to this question.

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Cutaneous extensibility in pseudo-xanthoma elasticum

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SUMMARY

In vivo skin elasticity was measured on unaffected forearm skin of twenty-five patients with pseudo-xanthoma elasticum and compared with age and sex-matched controls. The elastic modulus was raised in the recessive type I group and reduced in the dominant types I and II groups. No significant difference in skin thickness was found.

Pseudo-xanthoma elasticum (PXE) is an inherited disorder of connective tissue characterized by a flexurally-distributed lemon-yellow eruption and, on histopathological examination, by aggregations of elastic-staining material in the dermis. Intermittent claudication and gastro–intestinal bleeding may complicate the disease (Carlborg, 1944; Woo & Chandler, 1958) and angioid streaks and a degenerative choroidoretinitis which may be followed by blindness are common. It may be inherited as an autosomal recessive (Cockayne, 1933; McKusick, 1966) or as an autosomal dominant (Touraine, 1940). Cahill (1957) described transmission through three generations in what was clearly an autosomal dominant pattern.

Pope (1973) studied 140 affected individuals in England and Wales, and recognized four distinct groups, two of which were inherited as autosomal dominant (Pope, 1974a) and two as autosomal recessive characteristics (Pope, 1974b). The clinical characteristics of these groups are summarized in Table I, which shows their clinical features.

The basic defect of connective tissue in PXE was originally thought to reside in the collagen (Hall, Reed & Tunbridge, 1955), which underwent degenerative changes and in the process took on the staining properties of elastin. More recently, however, the structural defect has been attributed to elastin itself (Moran & Lansing, 1958) and an abnormal granular appearance of the 'amorphous' elastin component has been described (Ross, 1973).

Both collagen and elastin contribute to the mechanical properties of skin. Elastin is important in low strain conditions (elastic modulus* 3×10^5 pascals; Hass, 1942). Under conditions of higher strain the elastic behaviour of the skin depends upon collagen (elastic modulus approximately 1×10^8 pascals; Buck, 1954) and in particular upon the degree of inter-molecular cross-linking (Bailey, 1969).

Using the same technique for measuring skin elasticity, we have investigated the skin in two other hereditary diseases of connective tissue, the Ehlers–Danlos syndrome, in which an abnormal stress/

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* Elastic modulus (Young's modulus) is the stress divided by the strain.

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TABLE 1. Clinical characteristics of the genetic groups of PXE (After Pope, 1973)

	Dominant I	Dominant II	Recessive I	Recessive II
Number	12	52	54	3
Cutaneous				
Classical peau d'orange and flexural	12 (100%)	12 (24%)	40 (77%)	—
Macular eruption	—	35 (70%)	7 (13·9%)	—
General increase of extensibility	1 (8%)	33 (66%)	2 (3·9%)	—
General cutaneous PXE	—	—	—	3 (100%)
Vascular				
Angina	7 (56%)	—	—	—
Claudication	7 (56%)	—	—	—
Hypertension	9 (75%)	4 (7·8%)	10 (19·7%)	—
Haematemeses	1 (8%)	2 (3·8%)	8 (15·8%)	—
Ophthalmic				
Severe choroiditis	9 (75%)	4 (7·8%)	18 (35%)	—
Angioid streaks	4 (34%)	24 (47%)	24 (47%)	—
Transparent retina	—	6 (12%)	1 (1·9%)	—
Peau d'orange retina	—	4 (8%)	—	—
Prominent choroidal vessels	—	9 (18%)	—	—
Myopia	3 (24%)	24 (48%)	3 (5·8%)	—
High arched palate	—	27 (54%)	6 (11·9%)	—
Blue sclerae	1 (8%)	21 (41%)	5 (9·8%)	—
Loose jointedness	—	18 (35%)	3 (5·8%)	—

strain relationship was found during the initial phase of cutaneous extension (Grahame & Beighton, 1969), and cutis laxa, where dermal collagen was found to behave normally (Grahame & Beighton, 1971).

MATERIALS AND METHODS

Twenty-five patients were selected from the main series described in Table I for *in vivo* estimation of skin elasticity using an established suction cup method (Grahame, 1970). In this method a glass suction cup is applied to the dorsum of the right forearm and the distortion resulting from a series of graded negative pressures is measured by a method of water displacement. Using appropriate formulae (Tregear, 1966), a series of values for stress* and strain† is calculated and the gradient of the resultant stress/strain curve taken to represent the elastic modulus of skin. Skin thickness was measured with a Harpenden caliper (Tanner & Whitehouse, 1955) over the third right metacarpal shaft; similar measurements of skin thickness and elasticity were made on an identical number of age and sex-matched control subjects. The age and sex distribution between the genetic types is shown in Table 2.

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* Stress is defined as the force applied divided by the cross-sectional area.

† Strain is the distortion produced in response to stress and is defined as the increase in length divided by the initial length.

TABLE 2. Skin thickness and elasticity in PXE and controls

Type of PXE	Sex	Number per group	Mean age (yr)		Skin thickness (cm)		Skin elasticity*	
			Patients	Controls	Patients	Controls	Patients	Controls
Recessive I	M	1	66 (—)†	64 (—)	0·085 (—)	0·110 (—)	3·89 (—)	2·00 (—)
	F	6	40 (16)	41 (15)	0·102 (0·004)	0·103 (0·003)	3·42 (0·32)	2·27 (0·18)
Dominant I	M	5	46 (7)	46 (7)	0·125 (0·007)	0·112 (0·008)	1·60 (0·31)	2·14 (0·23)
	F	2	36 (2)	38 (3)	0·108 (0·107)	0·095 (0·005)	2·04 (0·35)	2·50 (0·50)
Dominant II	M	4	42 (7)	40 (6)	0·114 (0·013)	0·112 (0·009)	1·47 (0·20)	1·93 (0·28)
	F	7	27 (3)	26 (4)	0·110 (0·003)	0·103 (0·003)	1·76 (0·22)	2·50 (0·24)

* Elastic modulus expressed in pascals $\times 10^{-7}$.

† Values in brackets are the standard error of the mean.

RESULTS

Skin elasticity measurements showed that both the dominant type I and type II groups had lower elastic moduli than their control groups (Student's test for paired samples: $t = 2{\cdot}18$, $P<0{\cdot}05$ and $t = 2{\cdot}79$, $P<0{\cdot}02$ respectively). By contrast, the recessive type I group showed a significant increase in elastic modulus ($t = 3{\cdot}2$, $P<0{\cdot}25$; see Fig. 1). No significant differences of skin thickness were observed between any of the groups and their respective controls (Table 2).

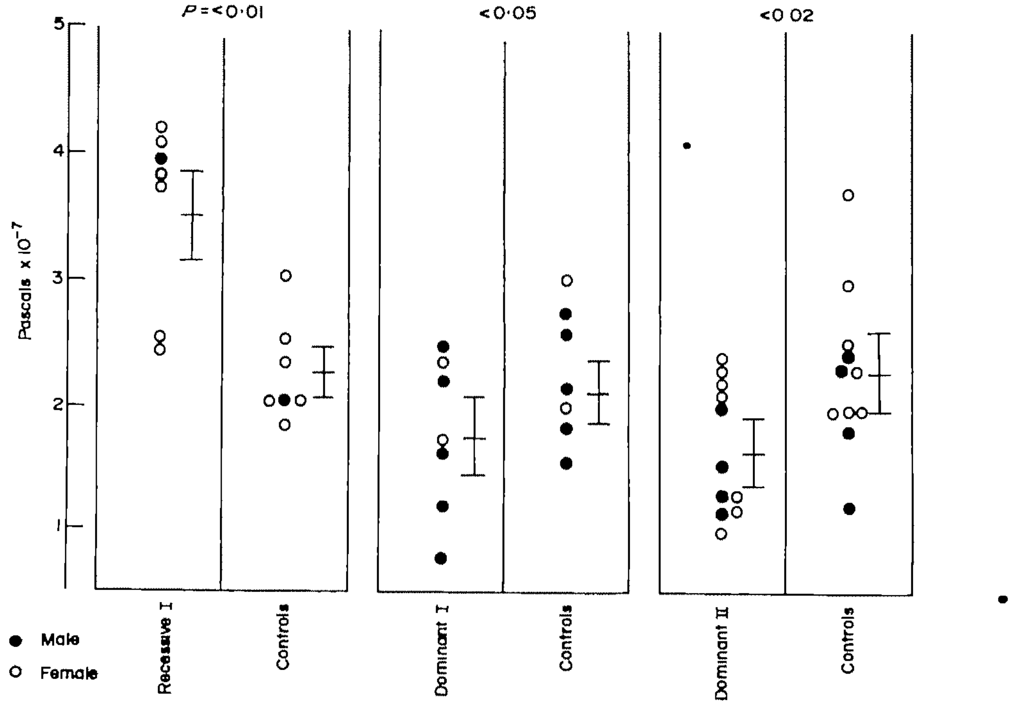

FIGURE 1. Elastic moduli of twenty-five patients with PXE grouped by their genetic pattern and compared with age and sex-matched controls.

DISCUSSION

The two autosomal dominant types of the disease showed a lower modulus of elasticity (i.e. a greater cutaneous extensibility) than control subjects, while the recessive type showed a higher elastic modulus. The tensile strength of collagen depends on the degree of inter-molecular cross-linking. This is supported by evidence of a reduction of the tensile strength of collagen in situations where a failure occurs in the development of lysine-derived aldehydes which are required for the formation of normal cross-links. Such a reduction in strength has been induced by lathyritic agents (Levene & Gross, 1959) and has also been observed in mice with a sex-linked defect of cross-linking in collagen and elastin (Rowe *et al.*, 1974).

There are objective differences in the cutaneous tensile strength of autosomal dominant and recessive forms of PXE. Studies of inter-molecular cross-linking are indicated and could show the biochemical basis of such heterogeneity.

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Chronic paronychia and psoriasis

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SUMMARY

Nineteen of fifty adult female patients with chronic paronychia and five of fifty adult female control dermatological patients suffered from psoriasis.

Chronic paronychia is a frequently encountered infection due to *Candida albicans* (Raubitschek, 1946; Hellier, 1955; Frain-Bell, 1957; Whittle, Moffat & Davis, 1959; Esteves, 1959; Marten, 1959; Stone & Mullins, 1963). In two previous publications (Ganor & Pumpianski, 1970; Ganor & Pumpianski, 1974) epidemiological aspects of this Candida infection have been described. However, no attention was given to host factors.

In capillaroscopic examinations of chronic paronychia characteristic changes were found (Ganor & Davis, 1974), which showed some similarity to those in certain persons suffering from psoriasis This leads to the question of whether a connection between chronic paronychia and psoriasis exists.

MATERIALS AND METHODS

Two groups of fifty adult female patients were studied. The first group consisted of fifty patients with chronic paronychia. The second group consisted of fifty adult female patients attending the outpatient clinic for other skin diseases (including psoriasis). They were chosen by being the first patients on each of fifty consecutive days who had not previously visited the outpatient clinic. Both groups were examined clinically for the presence of psoriasis at any skin site. All questionable lesions were excluded. The criteria for psoriasis were the presence of the Auspitz phenomenon in a suspected lesion or the presence of typical pitting of the nails. The majority were examined during winter. The age range of the chronic paronychia patients was 18–77 years (mean 44·5 years) and that of the controls was 22–77 years (mean 44·0 years).

RESULTS

Nineteen (38%) of the chronic paronychia patients had psoriasis; of these, five had pitting of a nail unaffected by paronychia. In five of the nineteen patients with psoriasis the hands were affected,

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but psoriasis was also present elsewhere. In three patients a small sacral lesion was found as the only manifestation of psoriasis. Five patients (10%) of the control group had psoriasis; in one patient pitting of one nail was the only manifestation of the disease. The control patients were attending the clinic because of eczema, tinea, pityriasis versicolor, brittle nails, perniosis, scabies, drug eruption, contact dermatitis, callosities, clavus, onychomycosis, solar keratosis, solar eczema, abscesses, pytiriasis rosea, leiomyoma, folliculitis, and two patients because of psoriasis.

The difference between the chronic paronychia and the control groups with regard to psoriasis was highly significant ($\chi^2 = 10{\cdot}8$; $P<0{\cdot}001$). No ethnic differences were found to account for the incidence of chronic paronychia as compared with controls, or for the incidence of psoriasis in the chronic paronychia group.

DISCUSSION

A parallel of the paronychia–psoriasis relationship is the so-called napkin psoriasis, in which *Candida albicans* is found more often in the lesions (Montes *et al.*, 1971) and in which psoriasis is detected more often in the relatives of these children (Andersen & Thomsen, 1971).

If the results presented here are confirmed in subsequent studies, several explanations may be given for the frequency of psoriasis in adult women with chronic paronychia.

(1) Nail involvement is present in many psoriatic patients (Zaias, 1967; Levin, De Wit & Ferrington, 1972). Thus, psoriasis enhances the possibility of *C. albicans* from the mouth or stools settling in an already injured nailfold (Ganor & Pumpianski, 1974).

The observation that 'comma' formation, a feature characteristic of chronic paronychia (Ganor & Davis, 1974), may be present also in psoriasis, especially when the nail is involved (Ganor & Davis unpublished observations) but, even when paronychia is absent, enhances the probability of this explanation.

(2) The changes from the oxidative to the glycolytic pathway in the epidermis (Braun-Falco & Petzoldt, 1967) or the increased turnover rate of the epidermis may enhance the growth of *C. albicans* in the nailfold.

(3) Impairment of peripheral blood flow contributes to chronic paronychia (Whittle *et al.*, 1959; Rook, Wilkinson & Ebling, 1972). In psoriasis there is a disturbance of the microcirculation in the skin (Gilje, O'Leary & Baldes, 1953; Holti, 1964; Ryan, 1969; Braverman, 1972).

Preliminary results in another non-matched group of fifty chronic paronychia and forty-two contro

TABLE 1. History of several diseases in unmatched adult female chronic paronychia and control patients

	Chronic paronychia (n = 50)	Control (n = 42)	Significance
Atopic disease	3	4	n.s.
Perniosis	17	6	$\chi^2 = 4{\cdot}7$; $P<0{\cdot}05$
Hyperhidrosis	13	7	n.s.
Varicosities	22	11	$\chi^2 = 3{\cdot}0$; $P<0{\cdot}1$ n.s.
Thrombophlebitis	9	1	$\chi^2 = 7{\cdot}2$; $P<0{\cdot}01$

n.s. = not significant.

patients supports a vascular aetiology. Perniosis, thrombophlebitis or varicose veins were anamnestically present more often in the chronic paronychia than in the control group (Table 1) which suggests that vascular diseases are more common in chronic paronychia patients. Also diabetes mellitus, with its angiopathy is more common in chronic paronychia (Hellier, 1955).

(4) Another explanation of these findings may be that dehydroepiandrosterone (DHA) deficiency predisposes to chronic paronychia. Although not looked for in perniosis and varicosities, DHA deficiency is common to psoriasis, thrombosis and diabetes (Holzmann *et al.*, 1970; Holzmann, Morsches & Hoede, 1973; Holzmann & Hoede, 1972).

(5) *C. albicans*, either in the lesion itself or at a remote site, may be a cause of the outbreak of clinical psoriasis in genetically predisposed people, a suggestion made by Fleischer & Wachowiak in 1925, and perhaps comparable to guttate psoriasis after streptococcal infection.

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'Pseudomorphoea': a side effect of subcutaneous corticosteroid injection

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SUMMARY

Three patients are reported who developed cutaneous atrophy in the upper arm following a single injection of a long-acting corticosteroid preparation into the deltoid region.

CASE HISTORY

All three patients were young women who reported to the dermatology clinic with similar clinical findings (Table 1). They had all received a single injection of corticosteroid into the deltoid region for the prophylactic treatment of hay fever.

TABLE I

Patient	Age	Sex	Date of injection	Dose	Date of appearance of lesion
1	23	F	June, 1971	40 mg methylprednisolone (Depo Medrone)	February, 1972
2	16	F	June, 1973	40 mg triamcinolone acetonide (Kenalog)	November, 1973
3	20	F	July, 1973	40 mg methylprednisolone (Depo Medrone)	October, 1973

Examination

In all three patients the lesion consisted of a patch of atrophic, depressed telangiectatic skin, with a mauve coloured periphery closely resembling the appearance of localized morphoea (Figs. 1a and b). The lesion had an irregular outline and an area of approximately 12 cm^2. Histologically these lesions showed remarkably little abnormality. There was, however, slight thinning of the epidermis and loss of rete pattern. The adnexal structures were higher in the dermis than usual, indicating dermal wasting.

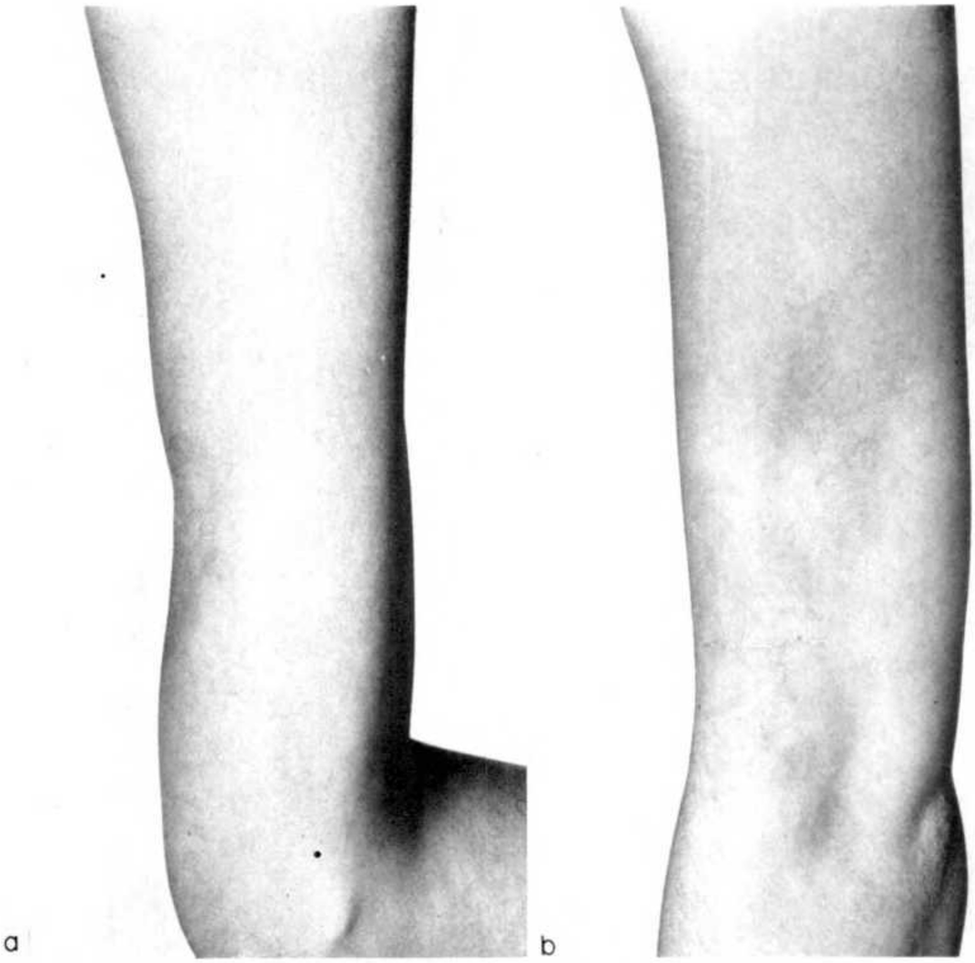

FIGURE 1. Left deltoid region showing (a) The depression; (b) Atrophy of the skin at the injection site.

Comment

It is well known that the connective tissue of skin is susceptible to the catabolic effect of corticosteroids whether administered systemically, topically or intracutaneously. It has been shown that glucocorticoids cause morphological changes in fibroblasts *in vitro* (Berliner & Ruhmann, 1967) and apparently inhibit protein synthesis in connective tissue. This report should serve to point out that injections of long acting steroids may produce a confusing clinical picture at the injection site, and in addition, illustrates a potential side effect of steroid therapy when these compounds are injected at this particular anatomical site.

Schetman, Hambrick & Wilson (1963) noted that triamcinolone-induced atrophy was dependent upon the anatomical level of the injection site as well as the local concentrations of the drug achieved. Medivestsky (1967) and Hefley *et al.* (1964) reported that cutaneous atrophy did not occur when steroid was given by deep intramuscular injection and therefore recommended the gluteal region for the site of intramuscular injection of corticosteroids. Jarratt, Spark & Arndt (1974) found that skin atrophy following intradermal injections of triamcinolone acetonide and triamcinolone diacetate was more marked than the atrophy seen following intradermal injection of betamethasone acetate–phosphate. They said that the glucocorticoid moiety in the triamcinolone compounds was released much more slowly into the injection site than in betamethasone acetate–phosphate therapy, prolonging the exposure of the injection site to the active triamcinolone salt.

There was a considerable time interval between the injection and the onset of the atrophy (Table 1) and the lesions continued to enlarge for several weeks after their appearance. In all three lesions spontaneous improvement was seen 3 months after their initial appearance. The resolution began as a filling in of the depressed area of skin and fading of the mauve discoloration. The lesion on patient 1 had completely healed without scarring 15 months after its appearance. The lesions on patient 2 and patient 3 continue to heal slowly.

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Pharmacology and Treatment

Effects of intensive application of retinoic acid on human skin

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SUMMARY

The daily application of 0·3% retinoic acid to the back produced an acute irritant dermatitis which resolved to a near-normal clinical appearance within 40 days despite continued daily exposure.

Hardened skin was markedly altered physiologically. The responses to DMSO, histamine and the histamine liberator, compound 48/80, were sharply enhanced. This reflected enhanced permeability resulting from reduction of the horny layer to less than one-half its normal thickness. Phototoxic and irritant substances produced exaggerated reactions owing to greater penetration. A paradoxical decrease in delayed sensitivity to streptodornase–streptokinase was attributed to enhanced clearance of the antigen.

The therapeutic repertoire of topical retinoic acid (vitamin A acid) has shown a remarkable increase over the past few years. Beneficial effects have been demonstrated in a variety of unrelated dermatoses. A review of its action and clinical application has been published (Ashton, Stevenson & Frenk, 1971). Apart from obvious alterations in the pattern of keratinization, these conditions share very little in common. Presumably, the correction of abnormal patterns of differentiation underlies the recently demonstrated usefulness of this agent in the suppression of induced tumours (Bollag & Ott, 1970; Prutkin, 1967; Bollag, 1971). For the most part, the mode of action in these multifarious disorders is unknown.

Christophers & Braun-Falco (1968) showed that the repeated application of 1% retinoic acid to guinea-pig ear skin for 7–10 days resulted in marked epidermal hyperplasia with an increase in the number of ^{3}H-thymidine labelled germinative cells. The stratum corneum became parakeratotic and the granular layer was thickened. In humans, Plewig, Wolff & Braun-Falco (1971) observed similar changes: marked thickening of the epidermis, hypergranulosis and a psoriasiform appearance.

We have found in the treatment of acne that daily application of retinoic acid produces a state of relative 'unresponsiveness' or hardening. This phenomenon has also been observed with other topical agents (McOsker & Beck, 1967). The object of this study was to evaluate the anatomical and behavioural changes induced in normal human skin by intensive exposure to retinoic acid. We hoped this might provide clues for explaining the moderating effect of this drug on so many different cutaneous disorders.

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MATERIALS AND METHODS

Subjects. Four white adult healthy prisoners between the ages of 25 and 35 years were volunteers.

Application of retinoic acid. 0·3% vitamin A acid was prepared in a vehicle consisting of 80 parts of absolute ethanol and 20 parts propylene glycol. Applications were made twice daily to one half of the back for a period of 40 days. The vehicle was applied to the other half.

Anatomical studies. The number of cell layers of the stratum corneum was determined by the alkali swelling technique (Christophers & Kligman, 1964) using cantharidin blisters.

Corneocyte counts were performed by the method of McGinley, Marples & Plewig (1969). Two 1 min detergent-scrubs of the surface were pooled and counted in a haemocytometer. Samples were obtained at the height of the inflammatory phase and at the end of 40 days.

Histology. Full thickness excision biopsies were obtained at the same time as above. The specimens were formalin fixed and stained with haematoxylin and eosin, PAS, Fontana's ammoniated silver, and Hale-orcein.

DNA-synthesizing cells were visualized by labelling the nuclei in the S-phase of the cell cycle with tritiated thymidine. 0·1 ml of saline containing 5 μCi of tritiated thymidine (specific activity 1·9 C/mM) was injected intradermally and the sites excised 45 min later. Specimens were fixed in formalin, sectioned (6 μm) and processed for radio-autography according to established procedures (Epstein & Maibach, 1965).

Physiological studies. The following comparisons were made on treated and untreated skin at the end of 40 days:

(i) Permeability. Dimethylsulfoxide (DMSO) was used to evaluate the permeability of the horny layer barrier. Twenty microlitres of 90% DMSO in water was pipetted into a 1·5 cm ring outlined by silicone grease. The penetration time was signalled by the appearance of 3–5 follicular weals.

(ii) Reactions to histamine and histamine liberator. Twenty microlitres of 1% histamine base in equal parts of absolute ethanol and water was applied under 1·5 cm discs of plastic film. Follicular weals were counted 30 min later. For histamine release, 2·3 cm squares of filter paper (No. 3, W. and R. Balston, Ltd) were saturated with an aqueous solution of compound 48/80 (0·07 mg/ml) and applied by iontophoresis to the mid-back for 3 min at a current density of 0·19 mA/cm^2. The number of follicular weals was estimated immediately afterwards.

(iii) Irritant chemicals. The following reagents were delivered in 0·05 ml quantities through tuberculin syringes to 1 cm squares of non-woven cotton cloth (Webril, Curity). (a) Croton oil, 5% in mineral oil; (b) kerosene (Ultrasene, Atlantic Richfield Co., Philadelphia, Pa.), 90% in mineral oil; (c) sodium lauryl sulphate (SLS) 2% in water. The patches were sealed to the skin under overlapping strips of Blenderm and secured with clear tape. Reactions were read 18 h later.

(iv) Phototoxic reactions. 40 μl of 25% coal tar distillate (DOAK Pharmacal Co., Inc., Jamaica, N.Y.) in absolute ethanol was pipetted onto 1·5 cm squares of skin outlined with clear adhesive tape (Johnson and Johnson). The application sites were then covered with plastic film and fastened with strips of Blenderm. The dressing was removed after 2 h, each site then received 3 min of long-ultraviolet light (LUV) from a xenon solar simulator, with the Schott WG345 filter to eliminate erythema producing waves. Reactions were graded 24 h later.

(v) Determination of MED's. The minimal erythema dose was determined, using a bank of five fluorescent FS20 sunlamps (Westinghouse Corp.) at a distance of 45 cm. One cm squares received increments of 15 s, starting with 60 s exposures.

(vi) Delayed allergic reactions. Three of the four subjects were sensitive to poison ivy and to varidase (streptokinase–streptodornase). These were challenged on the normal and treated skin to compare the magnitude of the delayed allergic response. 20 μl of 1:8 and 1:16 dilutions of poison ivy oleoresin (Hollister–Stier Laboratories) in acetone were applied to 1 cm squares of skin outlined with clear tape. The sites were covered with strips of plastic film (Saran) and secured with Blenderm and clear tape. The reactions were graded after 48 h.

The allergic reaction to varidase was provoked in the following way. A 2 cm square of skin was stripped to the glistening layer with scotch tape and covered with an equal sized double thickness square of Webril. 0·4 ml of varidase solution (1000 units/ml) in physiological saline was pipetted onto the cotton patch. The patches were fastened to the skin with Blenderm tape and clear tape. Reactions were graded in 48 h.

RESULTS

Clinical observations. Moderate erythema and scaling appeared after 3–4 days, and gradually increased in intensity to a peak in 12–15 days. The reactions were extremely inflammatory at that time and the sites sore and tender. The daily applications were continued as described. After about another week, the reactions began to wane. The irritant dermatitis gradually lessened in severity until by 40 days there remained only a slight erythema. The soreness and tenderness had disappeared completely. The treated skin appeared somewhat shiny and glistening but would not have appeared grossly damaged to the casual observer. We therefore judged the skin to be hardened by 40 days of treatment.

Anatomical studies. By 40 days, the stratum corneum was markedly reduced in thickness. The blisters from treated skin were friable and tore easily. Cell layer counts ranged between 7 and 10 as compared to 16 and 20 from the adjoining normal skin. The peak of the inflammatory phase, 15 days after the onset of treatment, was marked by an increase in the corneocyte count and in the percentage of nucleated cells (Table 1). These values reverted to near normal by 40 days.

TABLE 1. Corneocyte assay

	Peak inflammation				Hardening			
Subject	Total corneocytes*		(%) nucleated		Total corneocytes		(%) nucleated	
	Normal	Treated	Normal	Treated	Normal	Treated	Normal	Treated
1	79000	86000	2	2	60000	72000	0	2
2	89000	116000	0	20	62000	52000	1	3
3	75000	90000	2	32	58000	40000	2	3
4	52000	74000	0	16	48000	63000	1	0
Average	73750	91500	1	17·5	57000	56750	1	2

* Number of cells/cm^2.

Histology. At the peak of the dermatitis (2 weeks), the epidermis was thickened. Spotty or focal parakeratosis was seen. The granular layer was prominent but not increased in thickness. In the Malpighian layer, there were focal areas of cellular vacuolization and spongiosis. The germinative cell population was markedly increased as judged by the abundance of deeply staining basophilic cells with prominent nuclei, and the increased number of DNA synthesizing cells on radio-autographs

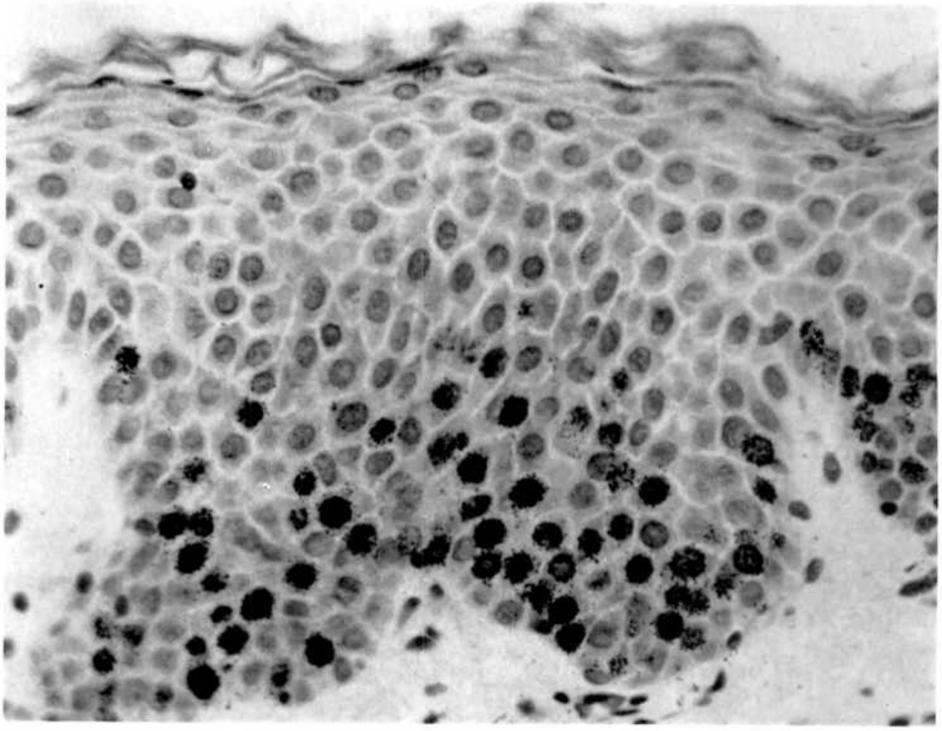

FIGURE 1. Radio-autograph, inflammatory phase; note acanthosis, intercellular oedema and increased number of DNA synthesizing cells. Compare with Fig. 2 (× 370).

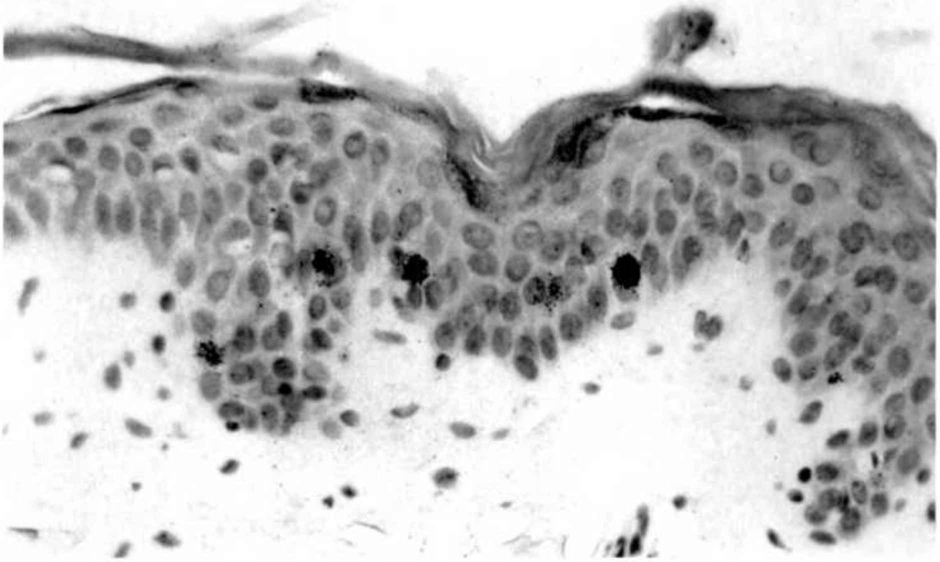

FIGURE 2. Radio-autograph, normal skin of the back from same subject as Fig. 1 (× 370).

(Figs. 1 and 2). The latter occupied the lowermost three cell layers of the rete mucosum. In the upper dermis, the vessels were prominent and engorged with red blood cells. A moderately intense perivascular round cell infiltrate was present throughout the upper and mid-dermis.

At 40 days, the epidermis was still thickened but less so than at 2 weeks. The stratum corneum was thin and orthokeratotic. A prominently thickened stratum granulosum composed of 4–5 cell layers was present. Germinative DNA-synthesizing cells were fewer in number compared with the 2 week specimens, but were still more than in normal skin. There did not appear to be any changes in collagen or elastic tissue. The vessels were much less dilated than at 2 weeks but an occasional one still showed a sparse mononuclear infiltrate. Silver positive melanin granules were uniformly and finely dispersed in the basal and suprabasal layers. The characteristic supra-nuclear caps of melanin in the basal cells of normal skin were absent (Fig. 3). Hale-orcein stains revealed a moderate increase in acid mucopolysaccharides in the papillary dermis.

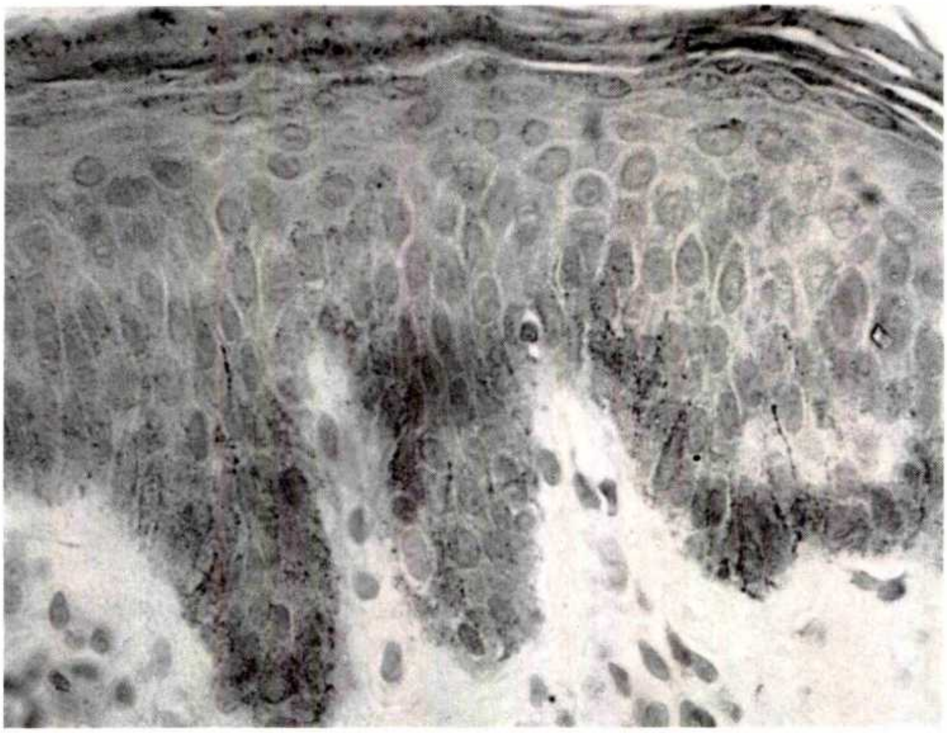

FIGURE 3. Hardened skin; note only minimal elongation of rete pegs, absence of spongiosis and dispersion of fine melanin granules among basal and parabasal cells. Compare with Fig. 4 (Fontana stain, × 505).

Permeability. After hardening, the penetration time of DMSO was dramatically shortened (Table 2). The average penetration time was 21 min and 15 s in the normal skin, as opposed to 9 min and 45 s in the treated skin.

Changes in reactivity. Three of the four subjects demonstrated an enhanced response to histamine and to the histamine liberator 48/80 (Table 3). The reactions also differed qualitatively. Weals appeared much sooner in the treated skin and the flare was considerably accentuated. The weals in treated skin also merged quickly.

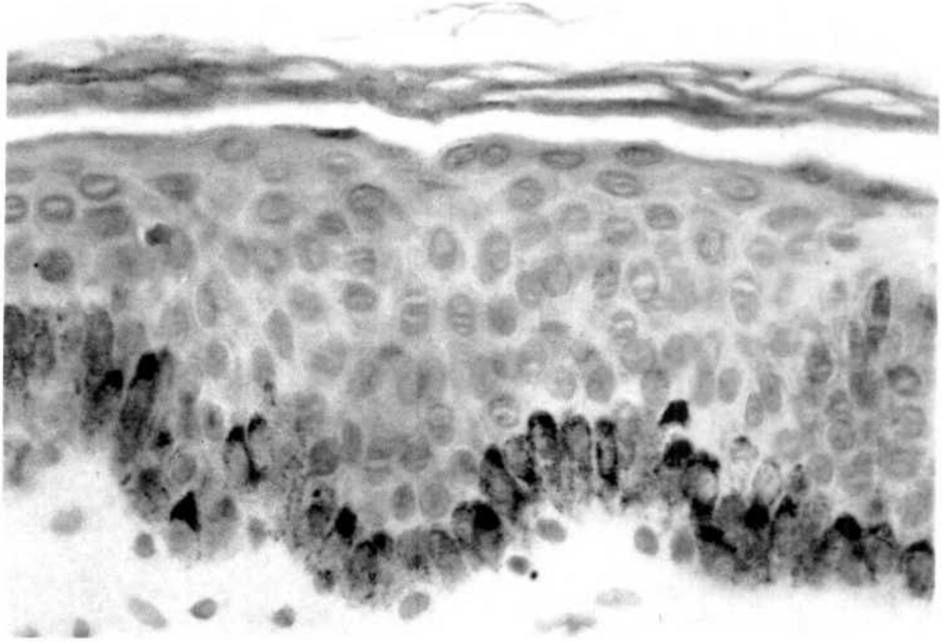

FIGURE 4. Normal skin of back from same subject as Fig. 3. Supra-nuclear condensation of melanin granules in basal cells (Fontana stain, × 505).

TABLE 2. Penetration time for DMSO

Subject	DMSO	
	Normal (min)	Treated (min)
1	20	12
2	15	7
3	25	10
4	25	10
Average	21·25	9·75

Irritant reactions. Irritant reactions, including phototoxic ones, were markedly accentuated on hardened skin (Table 4). SLS produced severely oedematous and crusted lesions on the treated skin. Croton oil produced not discrete pustules but confluent bullae. MED's were reduced from 90 to 75 s in the hardened skin of two subjects.

Delayed allergy. Two of the three subjects who were reactive to varidase showed a decrease in the delayed response in the treated skin. Only moderate erythema was present as opposed to marked erythema and oedema on the normal skin. No differences were discernible, however, following challenge with poison ivy oleoresin.

TABLE 3. Number of follicular weals in normal and retinoic acid treated skin

Subject	Compound 48/80		Histamine	
	Control	Treated	Control	Treated
1	29	25	0	15
2	25	60	4	Solid weal
3	23	60	8	10
4	15	35	4	Solid weal

TABLE 4. Response to irritants*

Subject	Tar Phototoxicity		SLS		Croton oil		Ultracene	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	1	3	1	3	2	4	3	4
2	1	2	1	3	2	4	4	4
3	1	3	1	3	2	4	3	4
4	2	3	1	2	2	3	3	4

* Reactions grades on a 1–4 scale. 1, erythema; 4, confluent bulla.

DISCUSSION

The intensive exposure of normal skin to retinoic acid for 40 days resulted in hardening. An acutely inflamed irritant dermatitis gave way in about 40 days to a near-normal appearance, despite continued daily applications.

Although hardened skin was not much altered clinically, various functional tests showed certain impairments. Characteristic microscopic changes accompanied the development of this state. The stratum corneum was reduced to about one-half its normal thickness. There was conspicuous hypergranulosis, moderate acanthosis and an increased germinative cell population in the epidermis. A prominent granular layer with darkly staining granules is a distinctive feature of retinoic acid irritancy. Acid mucopolysaccharides were moderately increased in the papillary dermis, and the blood vessels were more prominent.

The results of the various tests can be explained by increased permeability of the horny layer. DMSO and histamine promptly induced large weals in hardened skin. Phototoxic materials and primary irritants produced exaggerated responses. Sunburn was intensified. It seems likely that not only a thinner, but an abnormal, horny layer barrier enables these various substances to penetrate more rapidly. On the other hand, greater permeability tends to be counteracted by an increased dermal blood-flow. Substances diffuse inwards more readily, but once in the dermis, they are also more readily cleared. Thus, the attenuated delayed allergic response to streptodornase–streptokinase (varidase) was probably due to rapid removal of the antigen in the same way that injection of tuberculin along with histamine or other vasodilators gives lesser reactions (Magnusson, 1963).

Despite the fact that hardened skin showed acanthosis, hypergranulosis and an increased number of DNA synthesizing cells, hyperkeratosis did not occur. Retinoic acid differs in this respect from other irritant chemicals which characteristically cause the horny layer to thicken, even though it may be defective (McOsker & Beck, 1967). The marked thinning of the horny layer induced by retinoic acid stems from lack of cohesiveness between horny cells. The inability of cornified cells to stick together is one of the reasons why this drug prevents comedo formation in acne (Kligman, Fulton & Plewig, 1969). Suppression of keratin synthesis perhaps also contributes to the decreased cohesiveness. There is good evidence that retinoic acid does indeed inhibit keratinization. In cell cultures of post-embryonic human skin Karasek (1970) found that in low concentrations (0·1 mg/ml) cell growth was stimulated but keratin synthesis was inhibited. This had been directly visualized by electron microscopy of retinoic acid treated skin (Wolff, Christophers & Braun-Falco, 1970); both desmosomes and tonofilaments were decreased.

Our findings correlate well with earlier studies in providing some awareness of how retinoic acid exerts its salutary action in various hyperkeratotic conditions. Inhibition of keratinization and loss of cohesiveness are bound to lessen the excessive accumulation of horn in such conditions as palmoplantar keratoderma, pityriasis rubra pilaris and epidermolytic hyperkeratosis. As regards the ability of retinoic acid to combat neoplasms our findings are not informative; doubtless the drug influences the the differentiation of cells, possibly by its profound effect on lysosomal membranes (Reid & Jarrett, 1967; Dingle, 1963).

The capacity of retinoic acid to thin the barrier and increase percutaneous absorption suggests other clinical applications. The penetration of poorly diffusing substances could be enhanced. The greater effectiveness of a steroid–retinoic acid combination than the steroid alone in torpid, plaque-type psoriasis is at least partially explained by enhanced permeability (Macdonald & Fry, 1972; Orfanos, *et al.*, 1973). Likewise, we are finding that combinations of retinoic acid and antibiotics may render oral administration of the latter superfluous (unpublished observations).

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Treatment of solar keratoses of the extremities with retinoic acid and 5-fluorouracil

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SUMMARY

In contrast to solar keratoses of the face, twice daily application of 5% 5-fluorouracil cream was found to be incapable of destroying such lesions situated on the forearms and hands. Twice daily application of 0·1% retinoic acid was also ineffective.

Combined use of these agents eradicated keratoses in all twenty subjects with numerous keratoses of the forearms; in only two was the treatment ineffective for keratoses of the hands.

Topical 5-fluorouracil (5-FU) is an exceptionally effective means of eradicating solar keratoses of the face, destroying invisible lesions as well. It justifiably enjoys widespread use (Epstein, 1971). We and many others have observed that lesions on the backs of the hands and forearms respond poorly. Occlusive applications usually do not overcome this handicap. Ineffectiveness on the extremities is probably attributable to lack of penetration.

Retinoic acid (Tretinoin) is a drug with multifarious effects on epithelial structures (Logan, 1972; Jarrett & Spierman, 1970). It too has the capacity to destroy neoplasms such as solar keratoses and basal cell carcinomas (Klein *et al.*, 1966; Bollag & Ott, 1970). However, its efficacy in this regard is clearly less than that of 5-FU. The response is slower and often less complete. Retinoic acid has none the less been shown to cause regression of chemically induced epithelial tumours in mice and rabbits (Prutkin, 1967; Bollag, 1971). Moreover, vitamin A potentiates the cancer chemotherapeutic action of cytostatic drugs such as cyclophosphamide (Brandes *et al.*, 1966). 5-FU is a cytostatic drug while retinoic acid has the opposite effect.

The latter is known to speed up epidermal turnover and to lessen the cohesion between horny cells (Christophers & Braun-Falco, 1968). Desquamation is promoted and the horny layer becomes considerably thinner. We found a greater than 50% reduction in the number of cell layers of the stratum corneum after repeated application of retinoic acid (Kaidbey, Kligman & Yoshida, 1975). We also demonstrated increased penetration of vasodilators, dyes, and irritant chemicals. It seemed a likelihood therefore that retinoic acid could be used to promote the delivery of 5-FU to the neoplastic tissue. In addition tumour destruction would be likely to be enhanced if the drugs were used together, their modes of action being so completely different.

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The object of this paper is to show that the combination is highly efficaceous in eradicating solar keratoses of the hands and forearms.

MATERIALS AND METHODS

Two populations were studied—(1) Thirty-four men and women in military service, mostly young or middle-aged adults, with excessively sun-damaged skin and (2) an institutionalized group of indigent, mainly elderly persons ranging between 51 and 83 years of age. All had numerous keratoses of the face, hands and forearms.

Three tests were conducted—(1) 5% 5-FU cream (Efudix, Hoffman-La Roche) was applied twice daily to the face and to the backs of the hands and forearms of nine subjects for two weeks. (2) A solution of 0·05% retinoic acid (Retin-A, Johnson and Johnson) was applied twice daily to the same areas of five persons for 6–8 weeks. (3) In twenty subjects, 5% 5-FU cream was applied twice daily to one extremity. On the opposite side, 0·1% retinoic acid was liberally applied each morning and 5% 5-FU was applied in the evening. The daily applications of retinoic acid were stopped when a brisk inflammation developed usually in 8–14 days. After that, 5-FU cream was applied twice daily for an additional 3 weeks.

The results were recorded either as effective, signifying complete destruction of all keratoses, or ineffective if any remained. The aim was to eradicate all tumours; partial effects were interesting but not acceptable.

RESULTS

Study (1) *5-FU alone*

On the face 5% 5-FU was completely effective in all nine subjects (Plate 1). It was completely useless on the hands and was efficaceous in only two of nine patients on the forearms. For the most part, the cream behaved like a bland emollient, evoking no visible response.

Study (2) *Retinoic acid*

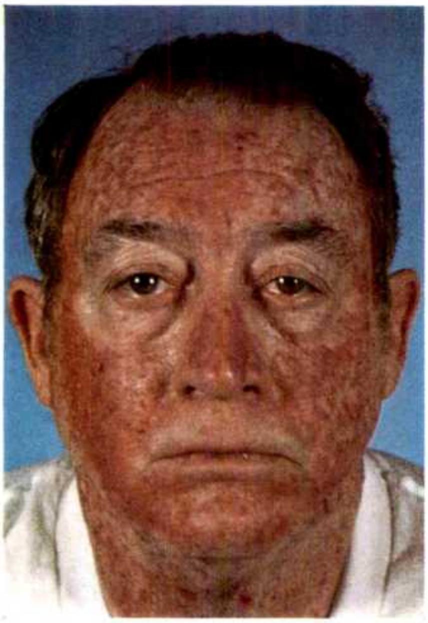
The 0·05% retinoic acid was effective on the face in three of the five subjects but on only one forearm. The hands were completely resistant. It should be noted that 6–8 weeks were required to achieve this unimpressive result. The skin became moderately irritated in all subjects within 1–2 weeks.

Study (3) *Combined therapy* vs. *5-FU alone*

Twice daily application of 5-FU was effective on the forearms in five of twenty subjects and in none on the hands (Plate 2). Combined therapy was effective on all twenty forearms and on eighteen hands. Visible and invisible lesions 'flared' in the familiar fashion.

CONCLUSION

Neither 5-FU nor retinoic acid alone was able satisfactorily to eradicate solar keratoses of the hands and forearms. The combination of the two provided an effective treatment. We are not the first to treat skin neoplasms with 5-FU and retinoic acid. Prutkin (1973) obtained almost complete regression



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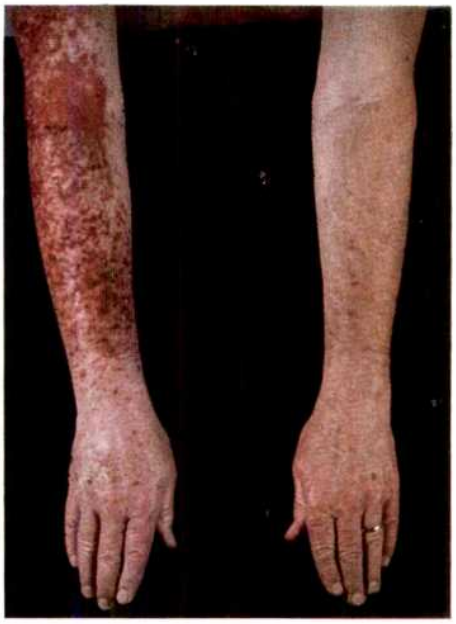

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PLATE 1. Inflammatory flare of facial keratoses 2 weeks after twice daily application of 5% 5-FU cream with complete eradication of lesions.

PLATE 2. Same patient as above. 5-FU cream was applied to the right forearm twice daily for 3 weeks with no visible reaction and without elimination of keratoses. Combined therapy was given to the left arm. Inflammatory activation of numerous invisible keratoses occurred with complete eradication of lesions.

of experimentally induced keratoacanthomas with this combination. At the biochemical level 5-FU has three modes of action. First, and undoubtedly the most important, is suppression of DNA synthetase (Cohen *et al.* 1958; Eagelstein, Weinstein & Frost, 1970). It may also be incorporated into a metabolically useless type of RNA (Dannenburg, Montag & Heidelberger, 1958). Finally it may prevent the utilization of uracil in synthesis of RNA (Heidelberger, 1965).

Retinoic acid, on the other hand, is not a cytostatic drug; it stimulates rather than represses cell proliferation. Its diverse effects are not explicable in biochemical terms. One can only describe what it does; everything else is speculation.

It functions as a regulator of epithelial differentiation, normalizing hyperplastic, metaplastic and precancerous tissue. Abnormal patterns of keratinization tend to be corrected. Thus retinoic acid has beneficial effects on disorders as different as ichthyotic and hyperkeratotic states, Darier's disease, acne, psoriasis, epithelial tumours and even warts. Labilization of lysosomes is one specific action which is thought to mediate certain of these effects, especially the dissolution of tumour cells (Lucy & Dingle, 1964; Dingle & Lucy, 1965; Brandes & Anton, 1966).

There is no reason to suppose that 5-FU would be any less effective in keratoses of the hands and forearms if it actually reached viable tissue. The diffusion of 5-FU into normal skin is slow, except where follicular shunts are numerous as on the face. A decisive effect of retinoic acid in this combination is to enable 5-FU to reach the target tissue. Of course, its own considerable anti-tumour action cannot be disregarded.

5-FU by itself was sometimes effective on the forearms but never on the dorsa of the hands, a region with a notably thicker horny layer. It was found that occlusive application could sometimes increase the effectiveness of 5-FU on the forearms, but never on the hands. A 5% 5-FU cream was used. Recently, Simmonds (1973) found 1% 5-FU to be as effective as 5% in solar keratoses. The lower concentration should also be evaluated in this combination.

Early on, it was found that little was to be gained by continuing retinoic acid once the keratoses had become intensely inflamed. Further experience, especially in the elderly, indicated that it may be appropriate to apply retinoic acid twice daily to induce inflammation earlier. Erythema and scaling merely signify that the horny layer barrier has been greatly reduced. Our present routine is to use morning and night applications of both agents, one after the other until the lesions exacerbate. 5-FU is then applied twice daily for another 2–3 weeks.

We are also evaluating a solution containing both agents; this is proving efficaceous in solar keratoses and in basal cell carcinomas for which conventional means of removal are inappropriate.

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Case Reports

Oestrogen-induced familial porphyria cutanea tarda

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SUMMARY

Two brothers with cutaneous hepatic porphyria are reported. One, aged 64 years, had his disease apparently precipitated by the oestrogen treatment given for carcinoma of the prostate.

A 64-year-old man, after 9 months' treatment with oestrogenic hormones for prostatic carcinoma, developed a cutaneous form of hepatic porphyria. The patient's brother, a year younger, has for the past 7 years been under observation for porphyria cutanea tarda (PCT). Both brothers have identical porphyrin excretion laboratory findings. Furthermore they both have diabetes mellitus.

Serial phlebotomies lessened the porphyrinuria and improved the skin symptoms. Re-administration of oestrogens, after a completed series of venesections, symptomlessly increased the porphyrinuria.

PCT after the administration of oestrogenic hormones is an infrequent occurrence. The importance of genetic factors and the mechanism are unknown.

CASE REPORT

A 64-year-old man was admitted to our department in June 1972. In April of that year he had a vesicular eruption on his fingers typical of PCT, which reappeared later several times.

Family history

The patient's younger brother aged 63 years was a diabetic. When he was 58 years old, PCT was diagnosed, but never treated. In January 1973 his urinary porphyrin excretion was: coproporphyrin (CP), 312·4 μg/24 h; uroporphyrin (UP), 2,864·6 μg/24 h. The Watson–Schwartz test for porphobilinogen (PBG) was negative. Faecal porphyrin excretion: CP, 43·8 μg/g dry wt, protoporphyrin (PP), 15·4 μg/g dry wt.

Past medical history

He had never been seriously ill. He used to drink one litre of beer a day but no spirits. In 1959 the first signs of nephrolithiasis appeared. Diabetes was diagnosed in 1968, and is being treated with a diet. In June 1971 prostatic hypertrophy was diagnosed. Prostatectomy was performed in mid-November

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1971. Microscopical examination confirmed the diagnosis of prostatic carcinoma. Post-operatively, the patient received Difostilben injections, as well as antibiotics and sulphonamides.

Clinical signs

Hypertension (190/105 mm Hg) and a slightly enlarged liver were discovered.

Laboratory investigations

ESR, 38 mm/h; cardiolipin, neg; blood count, blood urea, serum iron—all normal values. Urinalysis-glucose, positive; glycosuria, 0–9·0 g/24 h. Fasting blood glucose level, 111–128 mg%. Serum bilirubin, 0·8 mg%. Thymol turbidity test, 4·8 U. SGPT, 39·5 U/ml, SGOT, 46·5 U/ml. Total plasma proteins, 7·5 g% (γ globulin, 24%). BSP retention, 19·3%. Urinary porphyrins—CP, 177·1 μg/24 h; UP, 1209·1 μg/24 h. Watson–Schwartz test, negative. Faecal porphyrins: CP, 28·0 μg/g dry wt; PP, 49·2 μg/g dry wt. Liver biopsy histopathology: mild steatosis and haemosiderosis of hepatic cells with a slight fibrosis of portobiliary spaces.

Treatment

Since July 1972 the patient has been treated with phlebotomies and a total of 3600 ml of blood has been withdrawn. Porphyrinuria dropped to 238·3 μg of UP and to 30·0 μg of CP in 24 h.

The skin improved and in view of his progressing neoplasia the patient again was started on Difostilben. After 2 weeks the treatment was stopped, since laboratory studies revealed an increase in UP excretion to 1143·4 μg/24 h. Subsequently three venesections were performed a total of 1000 ml of blood being withdrawn. The uroporphyrinuria began to diminish and 3 weeks after the last venesection the uroporphyrins dropped to 490·0 μg/24 h.

DISCUSSION

Only a few cases of PCT induced by therapeutic administration of oestrogens have been described (Roenigk & Gottlob, 1970). The incidence of the disease no doubt is higher because it may remain clinically latent.

The problem of genetic predisposition in patients, who react to oestrogen treatment with clinical and laboratory signs of hepatic porphyria, remains open. But our patient is the only described case of PCT in its so-called 'pure' asymptomatic form with family incidence. This patient and his brother had increased values of urinary uro- and less so of coproporphyrin; a normal level of faecal porphyrin excretion; and no increased values of urinary porphyrin precursors. It appears from several reports that administration of oestrogens to 'normal' non-porphyric individuals fails to produce evidence of porphyria (Theologides, Kennedy & Watson, 1964; Copeman, Cripps & Summerly, 1966) and oral hormonal contraceptives (*British Medical Journal*, 1972) do not, in general, induce manifest porphyria in genetically unstigmatized individuals.

The studies of Granick (1966), Doss (1969) and of other authors suggest that oestrogens are porphyrogenic in chicken embryos. Repeatedly reproduced studies (Ippen, Aust & Goerz, 1972; Simon, Berkó & Dobozy, 1973) with experimental porphyria after hexachlorobenzene administration in rats (with a latent or with a manifest form of the disease) have proved that oestrogens can aggravate liver disorders either by rapidly producing manifest porphyric changes or by worsening existing ones.

Clinical reports to date concerning the influence of oestrogens on hepatic tissue are based both on laboratory abnormalities (BSP retention, activity of transaminases (Gallagher, Muller & Kappas, 1966; Kappas *et al.*, 1972)) and on microscopical findings (Kappas, 1967). The activity of the rate-limiting enzyme for porphyrin biosynthesis, δ-aminolaevulate (ALA) synthetase, is increased in the case of post-oestrogenic PCT (Levere, 1966). Other hepatotoxic substances, including alcohol, could also participate in these enzymopathic changes, but ALA-synthetase activity is not increased universally in all forms of hepatic porphyria (Kaufman & Marver, 1968).

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Milia occurring in lichen sclerosus et atrophicus

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SUMMARY

Large numbers of milia were seen in areas of skin affected with bullae in a 68-year-old woman who had extensive lichen sclerosus et atrophicus. Although milia are frequently seen in other sub-epidermal bullous disorders, they have not previously been reported in lichen sclerosus et atrophicus.

Lichen sclerosus et atrophicus is an uncommon condition of the skin which predominantly affects the genital region of middle-aged and elderly women. The skin of the trunk and limbs may also be affected with small white macular or atrophic plaques. Occasionally the lesions may spread rapidly to involve up to half the body surface. Bullous lesions are often seen histologically, but less frequently on clinical examination. A patient was seen with extensive lichen sclerosus et atrophicus and is being reported because there were several unusual features, including milia formation.

CASE REPORT

A 68-year-old Caucasian female first noticed white patches on her wrists at the age of 63 years. They were symptomless, but gradually spread to involve the skin of her neck, trunk (Fig. 1), upper arms and thighs. She was not aware of any abnormality in the perineum and had never had pruritus vulvae or ani.

On examination the individual lesions were characteristic of lichen sclerosus et atrophicus with tiny white porcelain-coloured macules and larger plaques which, in places, coalesced to produce large semitranslucent areas. Over the whole of her lower back the skin appeared to be thickened and could be moved over the underlying structures. In two areas on her back, examination showed a large number of milia within the area affected by the bullae (Fig. 2). Elsewhere there was no evidence of hyperkeratosis or of follicular plugging. The vulva and perianal skin showed the characteristic white pallor of lichen sclerosus et atrophicus with some telangiectasia and atrophy.

Two biopsies were taken from the patient's back. The first was through the edge of a typical lesion of lichen sclerosus et atrophicus and showed an atrophic epidermis with some liquefaction degeneration of the basal cells, homogenization of the collagen in the upper dermis and sub-epidermal bulla formation. There were a few inflammatory cells below the homogeneous band. The second biopsy, taken from an area of milia formation, showed several small keratinizing cysts in the upper dermis,

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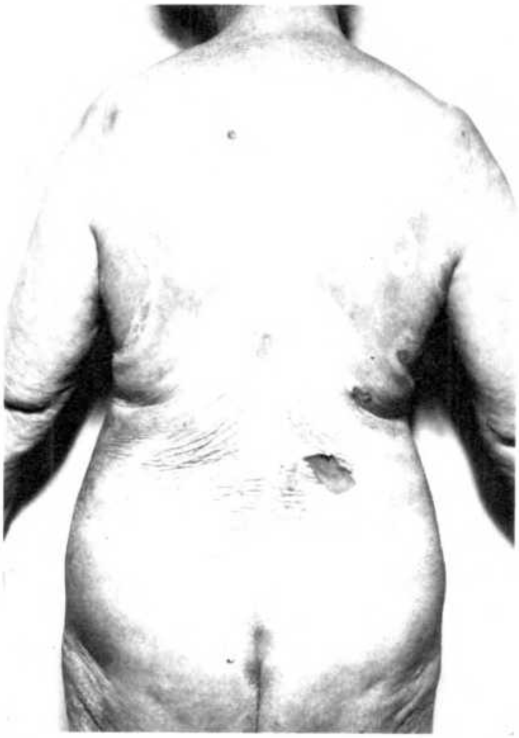

FIGURE 1. Patient's back showing extent of lichen sclerosus et atrophicus.

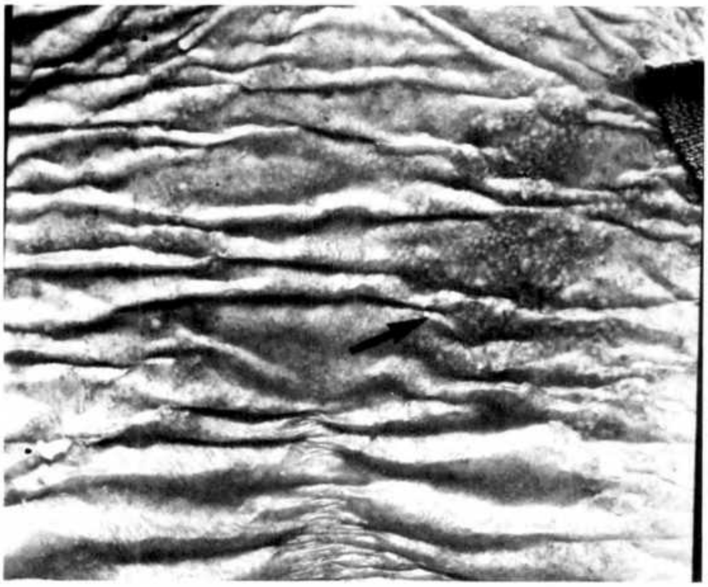

FIGURE 2. Close up of patient's back showing bullous area and large numbers of milia (arrowed).

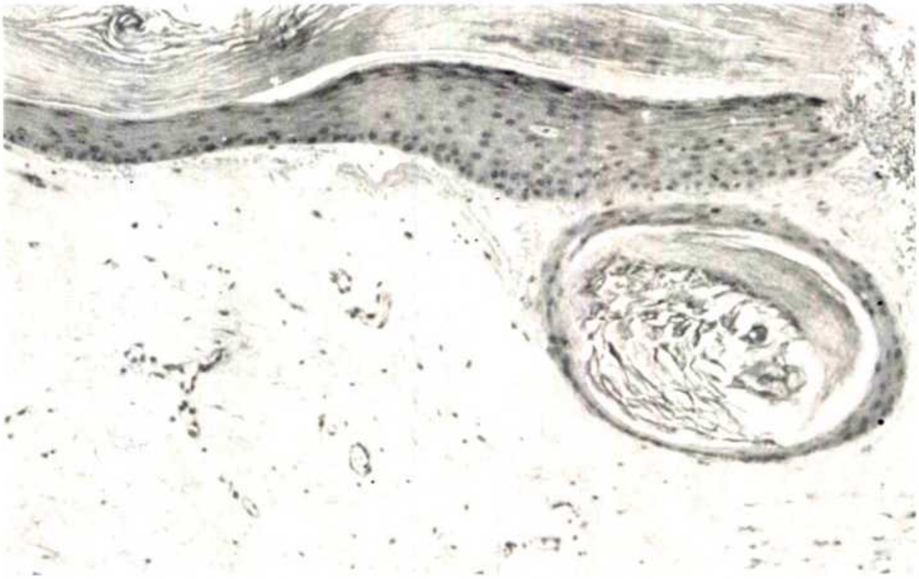

FIGURE 3. Histological features. Hyperkeratosis, sub-epidermal bulla formation and small keratinizing cyst in the upper dermis (H & E, × 100).

unconnected to any adnexal structure or the overlying epidermis (Fig. 3), in addition to the other histological changes of lichen sclerosus et atrophicus.

Further investigation

After this patient had been seen, histology sections from all the patients diagnosed as having lichen sclerosus et atrophicus and attending St John's Hospital for Diseases of the Skin during the last 10 years were examined. Eighty-eight sections from thirty-three patients were reviewed and those of five patients (15%) showed milia formation.

DISCUSSION

The usual sites to be involved with lichen sclerosus et atrophicus are the neck, upper trunk, flexor aspects of wrists and the ano-genital area (Kindler, 1953), and this patient showed extensive changes in these areas. The unusual features were, first, the rapid spread of the lesions to involve more than half the body surface in less than 5 years; second, the large areas of bulla formation on her back; and of third, the presence of milia.

In his long review, Wallace (1971) reported rapid spread of the lesions in only six (1·5%) of his 396 patients. The appearance of sub-epidermal bullae is a well recognized histological feature, but is less often observed on clinical examination. However, the patient reported by Myers & Dowling (1936) showed a very similar clinical picture to that seen in our patient, because he had a type of flat bulla with a thick horny roof. One of the characteristic histological findings in lichen sclerosus et atrophicus is diffuse hyperkeratosis with plugging of the hair follicles and sweat duct orifices, but milia formation has not been described.

Because milia are known to occur as secondary phenomena in other skin disorders which produce sub-epidermal bullae, such as pemphigoid, dystrophic epidermolysis bullosa and porphyria cutanea tarda, it would be unusual if they did not occur in lichen sclerosus et atrophicus. A milium may develop from any epithelial structure and on serial sections can usually be seen to be connected by a cord of undifferentiated epithelial cells to the parent structure whether this be hair follicle, sweat duct, sebaceous duct or epidermis (Epstein & Kligman, 1956). Any condition which produces damage to the adnexal structures may invoke milium formation.

In long-standing lichen sclerosus et atrophicus there may be atrophy of the hair follicles and sebaceous glands, although the sweat glands often remain intact. It is probable that the milia in this case were derived from the eccrine sweat ducts, for example, if these were ruptured when blistering occurred. Alternatively they could be produced by a mechanism similar to that which occurs in the type of hairless mice which have disappearing hair follicles. Montagna, Chase & Melaragno (1952) described a breed of homogeneous autosomal recessive hairless mice which grow a normal first pelage, but lose this by spontaneous epilation after the first few weeks of life. Why this happens is not known but once the hair is lost spherical epithelial cysts are formed in the dermis, and become larger and more numerous so that they eventually obliterate the dermis and adipose layer.

Whatever the cause, it is surprising that the occurrence of milia has not been described before in lichen sclerosus et atrophicus. It may be that they have been present but not noted in the past and that a careful clinical and histological examination will show that they are not infrequently present.

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Comment

LEAD AND THE SKIN

The physical and chemical properties of lead ensure that, provided supplies are not exhausted, it will have a future in technology to match its 6000 year past. No doubt too the persisting proximity of man and metal will continue to give rise to problems of toxicity.

Much work was done in the first half of this century to recognize and remove the more obvious sources of lead poisoning. Thus strict criteria are now laid down as to the maximum quantity of lead which may be contained in food, drinking water, paint for interior use and lead glazes; polyethylene and copper are used for plumbing, and even church roofs are more likely to be made of bitumenized felt than lead. In industry too, protective measures have resulted in florid lead poisoning becoming rare, although occasional accidents, such as those at Avonmouth, do occur (Windeyer, 1972).

There is, however, no room for complacency especially in view of the steadily increasing usage of lead. In 1940 the total United States consumption was 782 thousand tons and in 1968 1318 thousand tons (New York Academy of Sciences, 1972). The bulk of the increase has been used in petrol additives (50 to 262 thousand tons) and storage batteries (220 to 513 thousand tons) with a drop in the amount used as white lead in paint (from 66 to 6 thousand tons).

Attention has recently turned to the possibility of prolonged exposure to low doses of lead causing morbidity in the absence of the classical clinical features of poisoning. Every failed membership candidate knows that the Roman empire collapsed because the Romans drank acid wine from lead cups (Gilfillan, 1965); are we declining because we ingest some of the 100 thousand tons of lead puffed each year into the atmosphere of the Northern Hemisphere in the exhaust fumes of motor cars (Murozumi, Chow & Patterson, 1969)? The problem of airborne lead has been subject to intensive study (National Academy of Sciences, 1972) and the whole topic of lead poisoning has recently been reviewed in the informative monograph by Waldron & Stöfen (1974).

Lead is absorbed mostly through the lungs and gastrointestinal tract. Some is also absorbed through the skin but with inorganic compounds the amount is small. Sussman (1922) estimated that 100–200 μg of lead could penetrate 1 m^2 of skin per day but with the alkyl compounds used in petrol to prevent premature ignition the amount is much greater.

Shortly after the most widely used compound, tetraethyl lead (TEL), was first manufactured cases of toxicity began to occur; 139 cases being reported in 17 months, thirteen of them fatal. As a result manufacture was forbidden until attention to plant design produced greater safety (Hunter, 1969). Eldridge (1924) showed that significant absorption could occur through the skin; an observation which has been adequately confirmed (Kehoe, 1927; Kehoe & Thamann, 1931; Laug & Kunze, 1948). The hazard to those handling leaded petrol in a normal manner is probably small, (Kehoe, Thamann & Cholak, 1936) mainly because 95% of a dose applied to the open skin surface evaporates (Laug & Kunze, 1948). Cases of poisoning have been reported in inadequately protected workers cleaning storage tanks in which leaded fuel has been stored (Beattie, Moore & Goldberg, 1972).

Laug & Kunze (1948) studied the absorption of lead from various compounds painted on the shaved skin of rats and used the lead content of the kidneys as an index of absorption. Following TEL application there was a hundredfold increase and after lead acetate a threefold increase. Scarifying the skin enhanced absorption.

Martindale (1967) lists twenty two preparations containing lead, twenty for application to the skin surface and one, blended with cow's milk, for infusion into the rectum. Many are used for their astringent effect on exudative skin lesions and although absorption of lead through the intact skin surface may be slight there are no studies in humans assessing absorption of lead from these compounds through a broken skin surface.

Once absorbed about 95% of the lead is transported in the blood bound to lipids and lipoproteins on the erythrocyte membrane (Clarkson & Kench, 1958). TEL has a greater affinity for lipids than have inorganic compounds (Beattie *et al.*, 1972). In the plasma a little of the lead is in the free ionized state but most is bound to plasma proteins. Intracellular lead becomes firmly bound to the mitochondria (Castellino & Aloj, 1969), and, in common with other heavy metals, it has a strong affinity for sulphydryl groups. In the steady state approximately 90% of body lead is stored in the bones where it can replace calcium in crystalline structures.

Because of the richness of sulphydryl groups in keratin, hair contains a greater percentage of lead than any other tissue. This has resulted in its use as a biopsy material to assess lead exposure (Klevay 1973) but the amount varies with the age and sex of the patient so that great care is necessary in making comparisons (Petering, Yeager & Witherup, 1973). Renshaw, Pounds & Pearson (1972) have shown that the lead content of hair increases according to distance from the scalp, suggesting that lead is absorbed from the environment. This has been offered by Lockeretz (1973) as an explanation for some of the very high lead levels found when samples of hair from the late nineteenth and early twentieth century were examined (Weiss, Whitten & Leddy, 1972).

Excretion of lead occurs in the faeces and urine. Sweat contains a concentration similar to that in urine, but amounts in milk are small.

Lead poisoning *per se* does not produce skin lesions. The blue line which appears on the gums in some patients was first described by Grisolle in 1836 and later by Burton (1840) whose name is often eponymously attached to it (Hunter, 1969). The line is due to lead sulphide deposited subepithelially and formed by the interaction of hydrogen sulphide, from anaerobic organisms around the teeth, and circulating lead compounds. The appearance of the blue line under the electron microscope has recently been described (Hönigsmann, Jahn & Bauer, 1974). The blue line does not appear in persons with good dental hygiene, in the edentulous, or in infants before the eruption of teeth. A similar blue line may occur just within the anus, its presence being unrelated to the state of dentition.

An important aspect of the toxicity of lead is interference with haem biosynthesis. It acts by inhibiting enzymes, especially those which lie within the mitochondria to which it becomes attached, and produces an accumulation of the porphyrins or precursors formed at the previous stage in the chain. These effects are best summarized diagrammatically (Fig. 1).

It is tempting to compare lead poisoning with acute intermittent porphyria (Dagg *et al.*, 1965) which produces similar symptoms, but there are basic biochemical differences and the comparison should not be drawn too closely.

Three questions arise from the effects on porphyrin metabolism. Are the changes ever sufficient to cause photosensitivity? Can lead exacerbate a pre-existing abnormality? Can light influence the effect lead has on porphyrin metabolism?

Coproporphyrinuria has long been recognized as a feature of lead poisoning so it is perhaps surprising that no studies have been carried out to assess photosensitivity in sufferers. A recent paper (Allen *et al.*, 1974) reported three patients who were investigated because of blistering on the light exposed areas. One had severe overt lead poisoning and the others had biochemical evidence without classical symptoms. All three lived in areas with a highly plumbo-solvent water supply. It was suggested that lead may have provoked their photosensitivity but conclusive proof was lacking.

The cumulative or synergistic effects of lead and agents provoking porphyria have also been studied very little. There is an inverse relationship between blood lead and ALA dehydratase activity in the red cells (Hernberg & Nikkanen, 1970). This gives a clear indication of interference with porphyrin

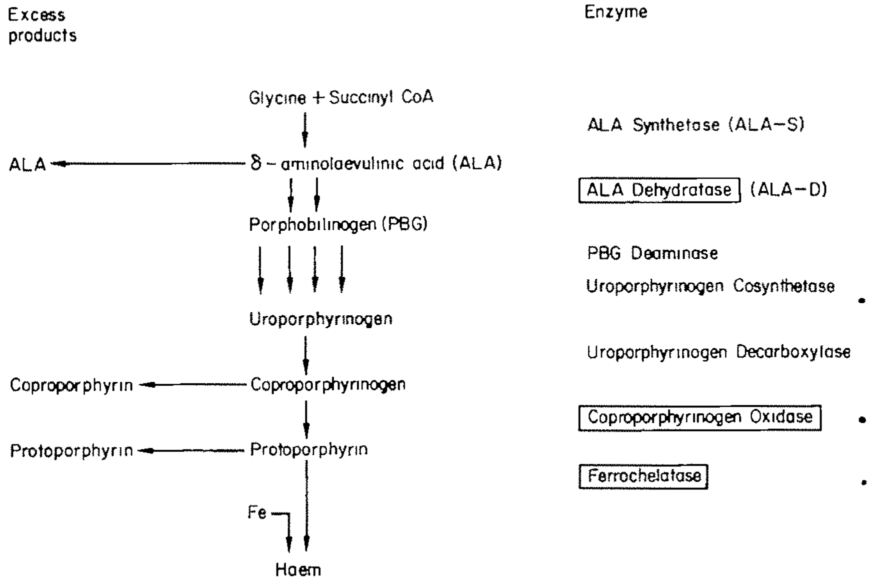

FIGURE 1. The effects of lead on Haem biosynthesis. Each vertical arrow represents one molecule; Enzymes boxed show the most marked inhibition.

metabolism even with what might be considered 'normal' exposure and therefore experimental studies designed to assess the interaction of lead and other porphyrinogenic agents will be of interest. Alcohol, for example, can induce ALA synthetase activity (Rubin *et al.*, 1970) and depresses ALA dehydratase activity (Moore *et al.*, 1971). The presence of lead, which further blocks ALA dehydratase, might produce an accumulation of ALA sufficient to cause behavioural disturbances such as were found in mice given ALA (McGillion, Moore & Goldberg, 1973). Alcoholics show an increased susceptibility to lead (Cramer, 1966) but Moore (1974) was unable to show a cumulative effect between alcohol and lead in rats. Wetterberg (1966) suggested that acute attacks in a patient with acute intermittent porphyria were provoked by ingesting fish with a high lead content, and Gheorghiu & Forsea (1968) attributed the onset of porphyria cutanea tarda to lead exposure in seventeen of their patients.

There is evidence that light itself may influence porphyrin metabolism. In plants ALA synthetase can be induced by light (Schneider, 1973) giving increased production of the main metalloporphyrin chlorophyll. It is a big jump from plants to mammals but Magnus, Janousek & Jones (1974) have shown increased amounts of ALA synthetase in the livers of rats made porphyric with 1, 4 dihydro-2, 6-trimethyl pyridine-3, 5-decarboxylate (D.D.C.) and exposed to light compared with controls and rats given D.D.C. and kept in the dark. Experimentally photosensitized and irradiated animals excrete increased amounts of coproporphyrin in their urine (Pimenta de Mello, 1949). Similarly Schwartz (1955) observed that rabbits poisoned with lead and kept in the dark excreted less coproporphyrin than if they were exposed to light but a proper experimental study is lacking. It has been suggested that the effect of light is to catalyse the formation of porphyrins and porphyrinogens from their precursors in the extracellular fluid (Burnett & Pathak, 1963).

There may be more published work on lead poisoning than on any other subject in toxicology but new pastures still remain for the interested research worker.

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Book Reviews

Organische und funktionelle Sexualerkrankungen. FRANZ FLECK and MARGARETE FLECK (1974) 2nd edn. Berlin: Veb Verlag Volk und Gesundheit. Pp. 485, 141 illustrations, 16 tables.

Dermatology, by strict definition the science of the skin, includes in practice the diseases of the oral and genital mucous membranes to an extent dependent on national tradition and the knowledge and inclination of the individual specialist. In some countries it includes much of the cosmetic work of the plastic surgeon and in some the dermatologist accepts responsibility for the burns unit. In some of the many countries in which the practice of dermatology includes venereology it has come also to include other diseases of the genitalia, and the growing subspecialty of sexology.

Professor Franz Fleck of Rostock and Professor Margarete Fleck of the Humboldt University of Berlin are perhaps best known to dermatologists for the very useful monograph on diseases of the hair which they published in 1962. In 1968 appeared the first edition of their text on 'Organic and Functional Sexual Disorders'. The volume under review is a revised and expanded edition of that work. It attempts to cover an enormous field. A short introduction summarizes the history of sexology, of venereology and sexual endocrinology and of aphrodisiacs. Sexual embryology, anatomy, physiology and endocrinology occupy the next 44 pages. There follow larger sections on venereal and non-venereal diseases of the genitalia (including conditions such as carcinoma of the cervix), fertility and sterility, diseases of the breast and sexual perversions.

Although many of these chapters deal with matters outside the practice of even the most broadly based dermatovenereologist, those that come within his scope present an effective and interesting summary which contains much that is of value; the 40 pages on gonadal influences on extragenital diseases for example are interesting and refer to work not well known in Western Europe or the United States.

Since it has reached a second edition it is evident that this book fills a need. It has, however, two serious defects. The first is that the authors have set themselves an impossible task in attempting to cover so much ground in under 500 pages and have been compelled to deal in too summary a fashion with many important conditions. The second defect is that although the authors' foreword is dated January 1973, the 20 page bibliography contains relatively few references of more recent date than 1965.

ARTHUR ROOK

Hospital Hygiene. ISOBEL M.MAURER (1974). London: Edward Arnold Ltd. Pp. 116. Price £4.50 bound, £2.25 paperback.

This book should be required reading for every ward sister, administrator and cleaning contractor. It sets out the philosophy and practice of hospital cleaning and disinfection and offers advice and 'Best Buys'. The author is a scientist in the Disinfection Reference Laboratory of the Public Health Laboratory Service and inevitably much of its information is laboratory based. Despite this the book is hospital orientated. In a discussion of the care of carpets in hospitals we find 'It is to be hoped that where a patient is distressed by a spillage, the first care of the staff will not be given to the carpet . . . .' The text is enlivened by cartoons and pictures of hospital life.

Perhaps the most directly useful chapter is that on 'A Policy for Sterilization and Disinfection'. Each area where disinfectants might be used is considered with a few brief statements on prevention and treatment; the range is from thermometers to animal houses. Hot water and anionic detergent is frequently recommended and elsewhere in the book the value of adequate cleaning is stressed as is the use of 'Heat—the best and cheapest disinfectant'.

If the reviewer has one minor criticism it is that brief statements on bacteriology have often led to dogma which might be challenged. Nevertheless this is a most valuable book; a copy should be lodged in every hospital ward and every laboratory.

W.S.Noble

The Normal Microbial Flora of Man. F.A.Skinner & J.G.Carr (Eds.) (1974) Society for Applied Bacteriology Symposium Series No 3. London and New York: Academic Press. Pp. xv+264. Price £6.00.

The papers in this book are those given at a Symposium of the Society for Applied Bacteriology in July 1973. Many of the papers will be of direct interest to those involved with skin and the others will have at least intrinsic interest. Following Sir Graham Wilson's introductory paper is a brief review of skin microbiology couched in ecological terms by Mary J. Marples. Many authors stressed this ecological approach and its value was spelled out by Hurley and her colleagues in a paper on the vaginal flora during pregnancy 'For the pathologist, clinicopathological correlation is the objective of ecological studies, since it allows him to lay down diagnostic criteria for diseases caused by microbes that may well form part of the endogenous flora in body sites other than that under study'. Woodroffe and Shaw follow Mary Marples with a detailed and well documented paper on natural control and ecology. The third, and last, paper on skin organisms is by R. R. Marples and details the work of the Philadelphia group on the effect of soaps, germicides and disinfectants.

The overall balance of the papers reflects not merely the growing interest in micro-ecology but also the extent of the effort put into such studies. Thus there are six papers on the gut flora, four papers on the mouth and respiratory tract, three on skin and one each on the vaginal flora and the normal viral flora. The paper by Burton *et al.* on tetracycline and the establishment and transfer of resistance in the gut neatly illustrates the difficulty of carrying out ecological studies in man. Whilst it is clear that resistance to antibiotics is transmitted in nature with apparent ease, it was necessary to give 1000 mg/day tetracycline orally for 9 days to achieve a reasonable, observable level of transfer in man. Nevertheless such attempts will continue to be of value for in this way it will be possible to obtain the clinicopathological correlations which underlie the need for these studies.

W.C.Noble

Sub-Clinical Lead Poisoning H.A.Waldron and D.Stöfen (1974) London: Academic Press. Pp. 224. Figs. 48. Price £5.50.

A full review of this book will not be included. It is, however, referred to in the Comment Article on page 715 of this issue.

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News and Notices

The Royal College of Physicians

The following Dermatologists have recently been elected to the Fellowship of the Royal College of Physicians, London: Dr P.W.M.Copeman, Dr C.Gresson, Dr A.W.Mackenzie, Dr R.D.G.Peachey, Dr E.L.M.Rhodes. Professor J.L.Turk was also elected.

Announcement

A special annual meeting of the International Society of Tropical Dermatology (ISTD) will be held in Guadalajara, Jalisco, Mexico, on Friday and Saturday, 12 and 13 December 1975. This two-day symposium will have to do with selected topics in cutaneous microbiology as they relate to the practice of clinical dermatology. The presenters will be specially invited experts, and the meeting will be structured into four one-half day sessions in each of the following areas: Bacteriology, Virology, Mycology and Parasitology. There will also be special cases presented under the direction of Dr Jose Barba Rubio, Professor of Dermatology, University of Guadalajara. Simultaneous English–Spanish translation will be provided.

Inscription costs for the meeting will be U.S. $20.00 for nationals of the host country and for members of the ISTD. Dermatologists, who are not members of the ISTD, are welcome and will be charged a registration fee of U.S. $50.00. Please address all correspondence to: Dr Orlando Canizares, Executive Secretary, International Society of Tropical Dermatology, 3 East 69th Street, New York, NY 10010, U.S.A.

Index

SUBJECTS

Acne vulgaris; plasma β-MSH levels in, 43
Action spectrum, erythemal, and DNA repair synthesis in xeroderma pigmentosum, 49
Adenocarcinoma of the large bowel and seborrhoeic keratoses, 449
Adenosine 3′,5′-cyclic monophosphate (cyclic AMP)
  and prostaglandins in epidermis, 381
  effects of acute ischaemia on, in skin, 249
  levels in normal appearing and lesional psoriatic epidermis, 619
  micro-determination of levels in human epidermis, dermis and hair follicles, 241
Adenosine triphosphatase (ATPase)-positive Langerhans cells in EDTA-separated sheets of mouse epidermis; cytochemical identification of, 523
Adenyl cyclase in psoriasis; further studies on, 255
  in psoriatic lesions; lack of response to catecholamine stimulation by, 619
Albinism in Nigeria, 485
Alopecia areata and smooth muscle antibodies, 389
  circular regrowth in (letter), 233
  traction; in Sikh boys (letter), 232
Amyloid and colloid or hyaline bodies; light and electron microscopic differentiation of, 637
Amyloidosis, primary cutaneous, with coeliac disease, 109
Angiogenic factor, tumour: associated with subcutaneous lymphoma, 273
Animal feed additives; contact dermatitis due to, 167
Anonychia with bizarre flexural pigmentation—an autosomal dominant dermatosis, 469
Antibiotics: tetracycline in the treatment of panniculitis (Rothmann-Makai), 351
Antibodies
  antireticulin, in dermatitis herpetiformis and adult coeliac disease, 37
  serum anti-tumour, and auto-antibodies in vitiligo, 395
  smooth muscle, and alopecia areata, 389
Antigens, epidermal, in lichen planus, 631
  liver, in systemic lupus erythematosus; modifying effect of autologous serum on leukocyte migration inhibition by, 401
Arsenic, absorption of, and its relation to carcinoma, 563
Ascorbic acid, high dose, in the management of thalassaemia leg ulcers: a pilot study, 339
Atopic disease and serum immunoglobulin-E, 17
  syndrome; ?earliest description of (letter), 359
Azaribine in the treatment of psoriasis: double-blind controlled crossover high-dose study, 199
Azathioprine and corticosteroids in the control of Wegener's granulomatosis, 461
Bacteria: bacteriological eco-system of the skin of children in an African tropical environment (Tanzania), 77
Bacterial infections: *M. fortuitum* and *M. chelonei*—fast growing mycobacteria, 453
Biochemistry
  adenosine 3′,5′-cyclic monophosphate (cyclic AMP); effects of acute ischaemia on, in skin, 249
    levels in normal appearing and lesional psoriatic epidermis, 619
    micro-determination of levels in human epidermis and hair follicles, 241
  adenyl cyclase in psoriasis; further studies on, 255
    system in psoriatic lesions; lack of response to catecholamine stimulation by, 619
  phospholipase A and lysophospholipase activity of the epidermis, 603
  plasma vitamin A levels in the dyskeratoses, 73
  prostaglandins and cyclic AMP in epidermis, 381
Book reviews
  Common Skin Diseases of Children in the Tropics, A.B.Shrank (colour slides), 117
  Funktionelle Dermatologie, G.Stuttgen and H.Schaefer, 116
  Gut-und Bösartige Erkrankgungen der Vulva. Klinik und Histologie. Band I. H.Grimmer, 482
  Hair Transplant Surgery, O.T.Norwood, 116
  Hospital Hygiene, I.M.Maurer, 720
  Immunological Aspects of Skin Diseases, ed. L.Fry and P.P.Seah, 480
  Insects and Other Arthropods of Medical Importance, ed. K.G.V.Smith, 481
  Lehrbuch der Haut Krankheiten und Venerischen Infectionen fur Studierende und Ärzte, T.Nasemann and W.Sauerbrey, 116
  Lupus Erythematosus. A review of the current status of Discoid and Systemic Lupus Erythematosus and their variants, 2nd edition, ed. E.L. Dubois, 236
  Microbiology of the Human Skin, W.C.Noble and Dorothy A.Somerville, 117
  Multiple Choice Questions in Dermatology, S.K. Goolmali, 115
  Organische und funktionelle Sexualerkrankungen, 2nd edition, F.Fleck and M.Fleck, 720
  Proceedings of the 14th International Congress of Dermatology, Padua/Venice, 22–27 May, 1972, ed. F.Serri and D.W.K.Cotton, 235
  Scleroderma (Progressive Systemic Sclerosis), A.J. Barnett, 361
  Skin Diseases in Children in Temperate Climates, A.B.Shrank (colour slides), 117
  Sub-Clinical Lead Poisoning, H.A.Waldron and D.Stöfen, 721

  The Herpes Viruses, ed. A.S.Kaplan, 236
  The Normal Microbial Flora of Man, ed. F.A.Skinner and J.G.Carr, 721
  Urticaria, R.P.Warin and R.H.Champion, 236
  Xanthoma Formation and Other Tissue Reactions to Hyperlipidemias, O. Braun-Falco, C.Keller and N.Zollner, 118
  Year Book of Dermatology 1974, ed. F.D.Malkinson and R.W.Pearson, 482

Cancer; the smell of, 215
Carcinoma; absorption of arsenic and its relation to, 563
  basal cell, benign inflammatory lesions and normal tissue; electrical potential difference between, 1
  of the large bowel and seborrhoeic keratoses, 449
Catecholamine stimulation; lack of response to, by the adenyl cyclase system in psoriatic lesions, 619
Cell culture
  growth inhibition in normal mammalian melanocytes *in vitro*, 291
  primary cell culture for biochemical studies of human keratinocytes, 305
Cell cycle of psoriasis (letter), 229, 230
Cell migration and DNA synthesis in organ culture of human skin, 375
Cells
  endothelial cells; search for microtubular inclusions in, in various skin diseases, 175
  epidermal cells, guinea-pig: Langerhans cells in tissue cultures of, 121
  keratinocytes, human: primary cell culture for biochemical studies of, 305
  Langerhans cells, ATPase-positive: cytochemical identification of, in EDTA-separated sheets of mouse epidermis, 523
    in tissue cultures of guinea-pig epidermal cells, 121
  leukocyte migration inhibition by liver antigens in systemic lupus erythematosus; modifying effect of autologous serum on, 401
  leukocytes, tuberculin-stimulated, from sarcoid and normal subjects; effect of transfer factor on the *in vitro* migration of, 535
  lymphocyte transformation test in allergic contact nickel dermatitis, 9
  melanocytes, normal mammalian; growth inhibition in, *in vitro*, 291
Chloride, sodium and hydrogen ion-specific electrodes: evaluation of in the study of the electrolyte contents of epidermal transudate and sweat, 511
Chronaxie, sensory; measurement of as a diagnostic procedure in scleroderma, 223
Civatte bodies, 637
Coal tar fractions; evaluation for use in psoriasiform diseases using the mouse tail test, 569, 575
Coeliac disease, adult, and dermatitis herpetiformis; antireticulin antibodies in, 37
  with primary cutaneous amyloidosis, 109
Colloid or hyaline bodies and amyloid: light and electron microscopic differentiation of, 637
Comment, 223, 355, 475, 593, 715
Complement deficiencies, 593
  components and properdin; dermal-epidermal deposition of, in systemic lupus erythematosus, 263
Concanavalin A and the surface coat of stratified squamous epithelium; interaction of, 27
Correspondence, 113, 229, 359, 479, 595
Corticosteroids
  and azathioprine in the control of Wegener's granulomatosis, 461
  for topical application; bio-assay of, 581
  hydrocortisone cream, 0·1% with vasoconstrictor activity and clinical effectiveness; new formulation of, 585
  injection, subcutaneous; 'pseudomorphoea'—a side effect of, 689
    triamcinolone acetonide treatment of psoriatic nail dystrophy; intradermal, with Port-o-Jet (letter), 479
Cutis laxa and post-inflammatory elastolysis, 183
Cytochemistry: identification of ATPase-positive Langerhans cells in EDTA-separated sheets of mouse epidermis, 523

Darier's disease: plasma vitamin A levels in, 73
Dequalinium chloride; microvascular response to local application of, 63
Dermatitis, atopic, 17
  contact
    due to additives in animal feed substances (ethoxyquin and halquinol), 167
    lymphocyte transformation test in allergic contact nickel dermatitis, 9
  herpetiformis
    and adult coeliac disease: antireticulin antibodies in, 37
    immunoglobulins in the skin in, and their relevance in diagnosis, 157
    iodine compounds and thyrotoxicosis (letter), 596
  napkin, psoriasiform; a follow-up study, 279
  nickel; hazards from prostheses, 287
Dermatophyte infection; areal extent of, 413
Dermatosis, acute febrile neutrophilic—see Sweet's syndrome
  autosomal dominant: anonychia with bizarre flexural pigmentation, 469
Dermis, human; micro-determination of cyclic AMP levels in epidermis, hair follicles and, 241
Diabetes mellitus; infections in (letter), 480
Dithranol; influence of treatment of paralesional skin with, on healing in psoriasis, 57
DNA repair synthesis and erythemal action spectrum in xeroderma pigmentosum, 49
  synthesis and cell migration in organ culture of human skin, 375
Drug eruption, fixed; due to paracetamol, 213
Dyskeratoses; plasma vitamin A levels in, 73

Eczema, atopic; humoral and cellular immunity in, 611
Elasticity of skin in pseudoxanthoma elasticum, 679
Elastolysis, post-inflammatory, and cutis laxa, 183
Electric potential difference between basal cell carcinoma, benign inflammatory lesions and normal tissue, 1

- Electron microscopy
  - a search for microtubular inclusions in endothelial cells in a variety of skin diseases, 175
  - electron microscopy in the identification of Langerhans cells in mouse epidermis, 527
  - interaction of Concanavalin A and the surface coat of stratified squamous epithelium, 27
  - light and electron microscopic differentiation of amyloid and colloid or hyaline bodies, 637
  - studies of changes at the migrating epidermal interface, 314
  - study of growth inhibition in normal mammalian melanocytes *in vitro*, 291
  - submicroscopic events during the acute photosensitivity flare in erythropoietic protoporphyria, 545
- Emotions and skin (II)—the conditioning of scratch responses in cases of lichen simplex, 407
- Endothelial cells; search for microtubular inclusions in, in various skin diseases, 175
- Environment, tropical African (Tanzania): bacteriological eco-system of the skin of children in, 77
- Enzymes
  - adenosine 3′,5′-cyclic monophosphate and prostaglandins in epidermis, 381
    - effects of acute ischaemia on, in skin, 249
    - micro-determination of levels in human epidermis, dermis and hair follicles, 241
  - adenyl cyclase in psoriasis; further studies on, 255
    - system in psoriatic lesions; lack of response to catecholamine stimulation by, 619
  - phospholipase A and lysophospholipase activity of the epidermis, 603
- Epidermal cells, guinea-pig: Langerhans cells in tissue cultures of, 121
  - electrolyte barrier; investigation of with Na⁺, Cl⁻ and pH ion-specific electrodes, 511
- Epidermis, human; micro-determination of cyclic AMP levels in dermis, hair follicles and, 241
  - migrating; the junctional zone beneath, 311
  - mouse: cytochemical identification of ATPase-positive Langerhans cells in EDTA-separated sheets of, 523
  - phospholipase A and lysophospholipase activity of, 603
- Epithelium, stratified squamous; interaction of surface coat of and Concanavalin A, 27
- Erythroderma, congenital ichthyosiform; oral retinoic acid in the treatment of, 343
- Ethoxyquin; contact dermatitis due to, 167

- Fat, from rat hair; removal and restitution of, 321
- 5-Fluorouracil:
  - and retinoic acid in the treatment of solar keratoses of the extremities, 703
  - and salicylic acid varnish; treatment of solar keratoses with, 85
  - in the treatment of common warts of the hands, 89
  - topical: controlled trial on the use of on viral warts, 93
- Fungal infections
  - dermatophyte infection; areal extent of, 413
  - *Trichophyton rubrum* and mycetoma (letter), 231
  - infections according to age, anatomical distribution and sex, 171

- German Dermatological Society: report on meeting in Graz, September 1974, (letter) 595
- Graft-versus-host reaction and lichen planus, 589, 591
- Granulomatosis, Wegener's: controlled by azathioprine and corticosteroids, 461
- Growth hormone (BGH) in the rat; sebotrophic action of, 325

- Hair (review)
  - I. The clinical significance of abnormal hair-slope patterns—trichoglyphics, 239
  - II. Racial and other genetic variations in hair form, 599
  - fat in the rat; removal and restitution of, 321
  - follicles, human; micro-determination of cyclic AMP levels in epidermis, dermis and, 241
  - loss; chemical aspects of, and its cosmetological significance, 355
- Halquinol; contact dermatitis due to, 167
- Herpes simplex, herpes zoster and viral warts in patients with secondary immune deficiencies and neoplasms, 625
- HL-A system and psoriasis, 427
- Hyaline or colloid bodies and amyloid; light and electron microscopic differentiation of, 637
- Hydrocortisone cream, 0·1%, with vasoconstrictor activity and clinical effectiveness; new formulation of, 585
- Hydrogen, sodium and chloride ion-specific electrodes; evaluation of in the study of the electrolyte contents of epidermal transudate and sweat, 511
- Hydroxyurea, idoxuridine and photodynamic inactivation in the treatment of warts, 97

- Ichthyosis: plasma vitamin A levels in, 73
- Idoxuridine, hydroxyurea and photodynamic inactivation in the treatment of warts, 97
- Immune deficiencies, secondary, and neoplasms; viral warts, herpes simplex and herpes zoster in patients with, 625
- Immunology
  - antireticulin antibodies in dermatitis herpetiformis and adult coeliac disease, 37
  - complement deficiencies, 593
  - dermal-epidermal deposition of complement components and properdin in systemic lupus erythematosus, 263
  - epidermal antigens in lichen planus, 631
  - humoral and cellular immunity in atopic eczema, 611
  - immunofluorescence studies of changes at the migrating epidermal interface, 312
  - immunoglobulin-E; serum level in atopic disease, 17
  - immunoglobulins in the skin in dermatitis herpetiformis and their relevance in diagnosis, 157
  - interaction of Concanavalin A and surface coat of stratified squamous epithelium, 27
  - modifying effect of autologous serum on leukocyte migration inhibition by liver antigens in systemic lupus erythematosus, 401

- serum anti-tumour antibodies and auto-antibodies in vitiligo, 395
- smooth muscle antibodies and alopecia areata, 389
- the HL-A system and psoriasis, 427
- viral warts, herpes simplex and herpes zoster in patients with secondary immune deficiences and neoplasms, 625

Inflammatory lesions, benign; basal cell carcinoma and normal tissue: electrical potential difference between, 1
Iodine compounds, dermatitis herpetiformis and thyrotoxicosis (letter), 596
Ischaemia, acute; effects of on cyclic AMP in skin, 249

Keratinocytes, human; primary cell culture for biochemical studies of, 305
Keratoses: seborrhoeic, with carcinoma of the large bowel, 449
- solar, of the extremities: treated with retinoic acid and 5-fluorouracil, 703
  - treatment of with a 5-fluorouracil and salicylic acid varnish, 85

Keratosis follicularis—see Darier's disease

Langerhans cells, ATPase-positive: cytochemical identification of, in EDTA-separated sheets of mouse epidermis, 523
- in tissue cultures of guinea-pig epidermal cells, 121

Lead and the skin, 715
Leishmaniasis, mucocutaneous, in guinea-pigs inoculated intravenously with *L. enriettii*, 663
Leser-Trélat; sign of, 449
Leukocyte migration inhibition by liver antigens in systemic lupus erythematosus; modifying effect of autologous serum on, 401
Leukocytes, tuberculin-stimulated, from sarcoid and normal subjects; effect of transfer factor on the *in vitro* migration of, 535
Lichen planus and graft-versus-host reaction, 589, 591
- epidermal antigens in, 631
- sclerosus et atrophicus in the Bantu (letter), 359
  - milia occurring in, 711
- simplex; conditioning of scratch responses in, 407

Light reactor, persistent; photochemistry of tetrachlorosalicylanilide and its relevance to, 143
- screening agents
  - chemical, 417
  - physical, 149

Lip; study of a new anomaly of the vermilion border of, 559
Lupus erythematosus, systemic; dermal-epidermal deposition of complement components and properdin in, 263
- modifying effect of autologous serum on leukocyte migration inhibition by liver antigens in, 401

Lymphocyte transformation test in allergic contact nickel dermatitis, 9
Lymphoma, subcutaneous; tumour angiogenic factor associated with, 273
Lysophospholipase and phospholipase A activity of the epidermis, 603

Melanocyte-stimulating hormone: plasma β-MSH levels in acne vulgaris, 43
Melanocytes, normal mammalian; growth inhibition in, *in vitro*, 291
Melanoma disseminatum, juvenile, 207
Microcirculation: effect of dequalinium chloride on, 63
Microscopy: light and electron microscopic differentiation of amyloid and colloid or hyaline bodies, 637
Milia occurring in lichen sclerosus et atrophicus, 711
Mites in the environment of cases of Norwegian scabies, 333
Mucosa, nasal: localization of blood-borne viable and non-viable particulate matter in, 475
Mycetoma and *Trichophyton rubrum* (letter), 231
Mycobacterium fortuitum and *Mycobacterium chelonei*—fast growing mycobacteria, 453

Nail dystrophy, psoriatic: intradermal triamcinolone acetonide treatment of, with Port-o-Jet (letter), 479
Neoplasms and secondary immune deficiencies; viral warts, herpes simplex and herpes zoster in patients with, 625
News and Notices, 120, 362, 484, 601, 722
Nickel dermatitis
- hazards from prostheses, 287
- lymphocyte transformation test in, 9

Oesophagus; tylosis following post-corrosive stricture of, 219
Ointment bases, bland; explanation for the 'placebo' effect of, 195
Organ culture: cell migration and DNA synthesis in human skin, 375

P values, in sales talk (letter), 359
Panniculitis (Rothmann-Makai), with good response to tetracycline, 351
Papules, piezogenic pedal (letter), 113
Paracetamol; fixed drug eruption due to, 213
Paronychia, chronic, and psoriasis, 685
Particulate matter, blood-borne viable and non-viable; endonasal localization of, 475
Patch testing in rabbit, using modified human patch test method: histological and visual assessment, 131
Phospholipase A and lysophospholipase activity of the epidermis, 603
Photobiology
- a study of chemical light screening agents, 417
- a study of physical light screening agents, 149

Photochemistry of tetrachlorosalicylanilide and its relevance to the persistent light reactor, 143
Photodynamic inactivation, hydroxyurea and idoxuridine in the treatment of warts, 97
Pigmentation, bizarre flexural, with anonychia: an autosomal dominant dermatosis, 469
Pityriasis rubra pilaris: plasma vitamin A levels in, 73
Plasma β-MSH levels in acne vulgaris, 43
- vitamin A levels in the dyskeratoses, 73

Polyp, adnexal; of neonatal skin, 659

Porphyria cutanea tarda, familial, oestrogen-induced, 707
  variegate: a case in an Indian, 347
Port-o-Jet: intradermal triamcinolone acetonide treatment of psoriatic nail dystrophy with (letter), 479
Prekeratin, epidermal, of bovine snout; heterogeneity of, 647
Properdin and complement components; dermal-epidermal deposition of, in systemic lupus erythematosus, 263
Prostaglandins and cyclic AMP in epidermis, 381
Protoporphyria, erythropoietic; submicroscopic events during the acute photosensitivity flare, 545
Protozoal infection: mucocutaneous leishmaniasis in guinea-pigs inoculated intravenously with *L. enriettii*, 663
Pseudo-monilethrix (letter), 113
'Pseudomorphoea'—a side effect of subcutaneous corticosteroid injection, 689
Pseudoxanthoma elasticum
  cutaneous extensibility in, 679
  historical evidence for the genetic heterogeneity of, 493
Psoriasiform diseases; evaluation of coal tar fractions for use in, using the mouse tail test, 569, 575
Psoriasis:
  age-of-onset of, and the relationship to parental psoriasis, 443
  and chronic paronychia, 685
  cell cycle of (letter), 229, 230
  double-blind controlled crossover high-dose study of Azaribine in, 199
  effect of the activity of on the response to autologous ground skin extract, 519
  further studies on adenyl cyclase in, 255
  healing in: influence of treatment of paralesional skin with dithranol on, 57
  HL-A system and, 427
  in children, 437
  lack of response to catecholamine stimulation by the adenyl cyclase system in lesions of, 619
  nail dystrophy in: intradermal triamcinolone acetonide treatment of, with Port-o-Jet (letter), 479

Retinoic acid
  and 5-fluorouracil in the treatment of solar ketatoses of the extremities, 703
  effects of intensive application of on human skin, 693
  oral; in the treatment of congenital ichthyosiform erythroderma, 343
Reviews, 239, 599
Rosacea; sebum excretion rate in, 541
Rothmann-Makai syndrome, treated with tetracycline, 351

Salicylic acid and 5-fluorouracil varnish; treatment of solar ketatoses with, 85
Sarcoidosis patients and normal subjects; effect of transfer factor on the *in vitro* migration of tuberculin stimulated white cells from, 535
Scabies, Norwegian: mites in the environment of, 333
Scleroderma; measurement of sensory chronaxie as a diagnostic procedure in, 223
Scratch responses in cases of lichen simplex; conditioning of, 407
Sebum excretion rate in rosacea, 541
  production in the rat; action of growth hormone (BGH) on, 325
Serum, autologous; modifying effect of on leukocyte migration inhibition by liver antigens in systemic lupus erythematosus, 401
Skin and lead, 715
  diseases, various: search for microtubular inclusions in endothelial cells in, 175
  elasticity in pseudoxanthoma elasticum, 679
  emotions and (II): conditioning of scratch responses in cases of lichen simplex, 407
  extract, autologous ground; effect of the activity of psoriasis on the response to, 519
  human; cell migration and DNA synthesis in organ culture of, 375
    effects of intensive application of retinoic acid on, 693
  manifestations associated with tumours of the brain, 675
  measurements of the surface of (thesis summary), 115
  normal, basal cell carcinoma and benign inflammatory lesions; electrical potential difference between, 1
  pig; effects of acute ischaemia on cyclic AMP in, 249
Sodium, chloride and hydrogen ion-specific electrodes; evaluation of in the study of the electrolyte contents of epidermal transudate and sweat, 511
Stratum corneum; method of evaluating the internal structure of (surfometry), 651
Surfometry; method of evaluating the internal structure of the stratum corneum, 651
Sweat and epidermal transudate; evaluation of sodium, chloride and hydrogen ion-specific electrodes in the study of the electrolyte contents of, 511
Sweet's syndrome: clinical spectrum of—report of 18 cases, 363

Tar oil acids; evaluation of for use in psoriasiform diseases, using the mouse tail test, 575
Tars, high and low temperature; evaluation of for use in psoriasiform diseases, using the mouse tail test, 569
Tetrachlorosalicylanilide; photochemistry of, and its relevance to the persistent light reactor, 143
Tetracycline in the treatment of panniculitis (Rothmann-Makai), 351
Thalassaemia leg ulcers; high dose ascorbic acid in the management of, 339
Thyrotoxicosis, iodine compounds and dermatitis herpetiformis (letter), 596
Tissue culture: Langerhans cells in tissue cultures of guinea-pig epidermal cells, 121
Transfer factor; effect of on the *in vitro* migration of tuberculin-stimulated white cells from sarcoid and normal subjects, 535

Transudate, epidermal, and sweat: evaluation of sodium, chloride and hydrogen ion-specific electrodes in the study of the electrolyte contents of, 511
Treatment
  ascorbic acid, high dose, in thalassaemia leg ulcers, 339
  Azaribine in psoriasis, 199
  dithranol on paralesional skin in psoriasis, 57
  5-fluorouracil and salicylic acid varnish in solar keratoses, 85
    in common warts of the hands, 89
    topical, in viral warts, 93
  hydroxyurea, idoxuridine and photodynamic inactivation using proflavin, in warts, 97
  induced tolerance to heat in localized heat urticaria, 191
  retinoic acid and 5-fluorouracil in solar keratoses of the extremities, 703
    oral, in congenital ichthyosiform erythroderma, 343
  triamcinolone acetonide, intradermal, in psoriatic nail dystrophy (letter), 479
Triamcinolone acetonide treatment of psoriatic nail dystrophy; intradermal, with Port-o-Jet (letter), 479
Trichoglyphics: the clinical significance of abnormal hair-slope patterns, 239
Trichophyton rubrum and mycetoma (letter), 231
  infections according to age, anatomical distribution and sex, 171
Tumour angiogenic factor associated with subcutaneous lymphoma, 273
Tumours, brain; skin manifestations associated with, 675
Tylosis following post-corrosive stricture of the oesophagus, 219

Ulcers of leg, in thalassaemia; high dose ascorbic acid in the management of, 339
Ultraviolet radiation; changes in the skin in erythropoietic protoporphyria following, 545
Urticaria, localized heat: treated by inducing tolerance to heat, 191
  solar, 101

Verruca vulgaris—see Warts
Vitamin A acid—see Retinoic acid
  levels in plasma, in the dyskeratoses, 73
Vitiligo; serum anti-tumour antibodies and auto-antibodies in, 395

Warts; anti-viral treatment of, 97
  common, of the hands: 5-fluorouracil in the treatment of, 89
  viral: controlled trial on the use of topical 5-fluorouracil on, 93
    herpes simplex and herpes zoster in patients with secondary immune deficiencies and neoplasms, 625

Xeroderma pigmentosum; erythemal action spectrum and DNA repair synthesis in, 49

AUTHORS

Abell, E., Nishikawa, T.; Marks, R. and: the junctional zone beneath migrating epidermis, 311
Abell, E., Nishikawa, T., Marks, R. and Ramnarain, D., epidermal antigens in lichen planus, 631
Adachi, K., Halprin, K.M., Levine, V.; Yoshikawa, K. and: cyclic AMP in skin: effects of acute ischaemia, 249
Adachi, K., Halprin, K.M., Levine, V.; Yoshikawa, K. and: micro-determination of cyclic AMP levels in human epidermis, dermis and hair follicles, 241
Adachi, K., Halprin, K.M., Levine, V.; Yoshikawa, K. and: on the lack of response to catecholamine stimulation by the adenyl cyclase system in psoriatic lesions, 619
Adachi, K., Yoshikawa, K., Halprin, K.M. and Levine, V., prostaglandins and cyclic AMP in epidermis, 381
Afifi, A.M., Ellis, L., Huntsman, R.G. and Said, M.I., high dose ascorbic acid in the management of thalassaemia leg ulcers—a pilot study, 339
Alexander, J.O'D.; Douglas, W.S. and: dermatitis herpetiformis, iodine compounds and thyrotoxicosis (letter), 596
Allen, B.R., Moore, M.R. and Hunter, J.A.A., lead and the skin, 715
Anderson, Jeannette, Dave, V.K., Usher, N.; Woodrow, J.C. and: the HL-A system and psoriasis, 427
Andreev, V.C. and Petkov, I., skin manifestations associated with tumours of the brain, 675
Asensio, J., Garcia-Calderon, P., De Moragas, J.M.; Gimenez-Camarasa, J.M. and: lymphocyte transformation test in allergic contact nickel dermatitis, 9
Augustine, S.M., Yesudian, P., Kamalam, A., Premalatha, S.; Thambiah, A.S. and: tylosis following post-corrosive stricture of the oesophagus, 219

Baart De La Faille, H., Rottier, P.B. and Baart De La Faille-Kuyper, E.H., solar urticaria, 101
Baker, H., Sattar, H., Casey, T.; Grice, Katherine, and: an evaluation of Na$^+$, Cl$^-$ and pH ion-specific electrodes in the study of the electrolyte contents of epidermal transudate and sweat, 511
Bayles, Margaret A.H.; Bentley-Phillips, B. and: pseudo-monilethrix (letter), 113
Bentley-Phillips, B. and Bayles, Margaret A.H., pseudo-monilethrix (letter), 113
Bettley, F.R. and O'Shea, J.A., the absorption of arsenic and its relation to carcinoma, 563
Blank, F. and Mann, S.J., *Trichophyton rubrum*

infections according to age, anatomical distribution and sex, 171

Bleeker, J.J., intradermal triamcinolone acetonide treatment of psoriatic nail dystrophy with Port-o-Jet (letter), 479

Britten, A.Z.; Wrench, Rosanne, and: evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test. I. High and low temperature tars and their constituents, 569. II. Tar oil acids, 575.

Burrows, D., contact dermatitis in animal feed mill workers, 167

Burton, J.L., taking P values out of sales talk (letter), 359

Burton, J.L., Goolamali, S.K., Plummer, N.A., Shuster, S.; Thody, A.J. and: plasma β-MSH levels in acne vulgaris, 43

Burton, J.L., Pye, R.J., Meyrick, Gay, and Shuster, S., the sebum excretion rate in rosacea, 541

Bussel, Annette, Didier-Jean, Liliane, Gluckman, Eliane; Saurat, J.H. and: graft versus host reaction and lichen planus-like eruption in man, 591

Caldwell, I.W., White, J.E.; Liddell, K. and: seborrhoeic keratoses and carcinoma of the large bowel, 449

Canti, G., Watson, B.W.; Woodrough, R.E. and: electrical potential difference between basal cell carcinoma, benign inflammatory lesions and normal tissue, 1

Capetanakis, J., juvenile melanoma disseminatum, 207

Carruthers, C.; Wissler, F. and: heterogeneity of bovine snout epidermal prekeratin, 647

Carslaw, R.W., Dobson, R.M., Hood, A.J.K. and Taylor, R.N., mites in the environment of cases of Norwegian scabies, 333

Casey, T., Sattar, H., Baker, H.; Grice, Katherine, and: an evaluation of Na$^+$, Cl$^-$ and pH ion-specific electrodes in the study of the electrolyte contents of epidermal transudate and sweat, 511

Chan, H.L., panniculitis (Rothmann-Makai), with good response to tetracycline, 351

Chlumsky, J.; Malina, L. and: oestrogen-induced familial porphyria cutanea tarda, 707

Clark, M.L., Kumar, Parveen, Marks, R., Johnson, G.D.; Lancaster-Smith, M. and: antireticulin antibodies in dermatitis herpetiformis and adult coeliac disease, 37

Cooper, J.R.; Cruickshank, C.N.D. and: Langerhans cells in tissue cultures of guinea-pig epidermal cells, 121

Cormane, R.H.; Eriksen, L. and: oral retinoic acid as therapy for congenital ichthyosiform erythroderma, 343

Cruickshank, C.N.D. and Cooper, J.R., Langerhans cells in tissue cultures of guinea-pig epidermal cells, 121

Crutcher, W.A. and Moschella, S.L., double-blind controlled crossover high-dose study of Azaribine in psoriasis, 199

Dave, V.K.; Stankler, L. and: the influence of treatment of the paralesional skin with dithranol on healing in psoriasis, 57

Dave, V.K., Usher, N., Anderson, Jeannette; Woodrow, J.C. and: the HL-A system and psoriasis, 427

Davies, A.K., Hilal, N.S., McKellar, J.F. and Phillips, G.O., photochemistry of tetrachlorosalicylanilide and its relevance to the persistent light reactor, 143

De Bats, A., Rhodes, E.L.; Park, J.R. and: the effect of transfer factor on the *in vitro* migration of tuberculin stimulated white cells from sarcoid and normal subjects, 535

De Moragas, J.M., Garcia-Calderon, P., Asensio, J.; Gimenez-Camarasa, J.M. and: lymphocyte transformation test in allergic contact nickel dermatitis, 9

Delaney, T.J.; Tan, R.S.H. and: circular regrowth in alopecia areata (letter), 233

Didier-Jean, Liliane, Gluckman, Eliane, Bussel, Annette; Saurat, J.H. and: graft versus host reaction and lichen planus-like eruption in man, 591

Dobson, R.M., Hood, A.J.K., Taylor, R.N.; Carslaw, R.W. and: mites in the environment of cases of Norwegian scabies, 333

Dogliotti, M., lichen sclerosus et atrophicus in the Bantu (letter), 359

Donald, D., Robbie, Rosalene B., Gray, Elizabeth S., Horne, C.H.W.; Main, R.A. and: smooth muscle antibodies and alopecia areata, 389

Douglas, W.S. and Alexander, J.O'D., dermatitis herpetiformis, iodine compounds and thyrotoxicosis (letter), 596

Dreyfus, B., Revuz, J., Rochant, H., Mannoni, P.; Touraine, R. and: graft versus host reaction and lichen planus, 589

Ebling, F.J., Ebling, Erika, Randall, Valerie, and Skinner, J., the sebotrophic action of growth hormone (BGH) in the rat, 325

Ebling, F.J., and Skinner, J., the removal and restitution of hair fat in the rat, 321

Ebner, H. and Gebhart, W., light and electron microscopic differentiation of amyloid and colloid or hyaline bodies, 637

Elkomy, M., Hanna, N.; Mohareb, R. and: study of a new anomaly of the vermilion border of the lip, 559

Ellis, L., Huntsman, R.G., Said, M.I.; Afifi, A.M. and: high dose ascorbic acid in the management of thalassaemia leg ulcers—a pilot study, 339

Eriksen, L. and Cormane, R.H., oral retinoic acid as therapy for congenital ichthyosiform erythroderma, 343

Finn, O.A., MacKie, Rona M., McQueen, A., MacSween, R.N.M.; Woolfson, H. and: serum anti-tumour antibodies and auto-antibodies in vitiligo, 395

Finn, O.A.; Neville, E.A. and: psoriasiform napkin dermatitis—a follow-up study, 279

Flaxman, B.A. and Harper, R.A., primary cell culture for biochemical studies of human keratinocytes, 305

Forbes, I.J., Reid, J.G.; Grove, D.I. and: humoral and cellular immunity in atopic eczema, 611

Frain-Bell, W.; MacLeod, T.M. and: a study of chemical light screening agents, 417

Frain-Bell, W.; MacLeod, T.M. and: a study of physical light screening agents, 149

Fry, L., Hamilton, Susan; Goodwin, P. and: cell cycle of psoriasis (letter), 230

Fry, L.; Seah, P.P. and: immunoglobulins in the skin in dermatitis herpetiformis and their relevance in diagnosis, 157

Ganor, S., chronic paronychia and psoriasis, 685

Garcia-Calderon, P., Asensio, J., De Moragas, J.M.; Gimenez-Camarasa, J.M. and: lymphocyte transformation test in allergic contact nickel dermatitis, 9

Garretts, M., Brief aus Graz (letter), 595

Gaylarde, P.M. and Sarkany, I., cell migration and DNA synthesis in organ culture of human skin, 375

Gebhart, W.; Ebner, H. and: light and electron microscopic differentiation of amyloid and colloid or hyaline bodies, 637

Giannelli, F.; Ramsay, C.A. and: the erythemal action spectrum and DNA repair synthesis in xeroderma pigmentosum, 49

Gimenez-Camarasa, J.M., Garcia-Calderon, P., Asensio, J. and De Moragas, J.M., lymphocyte transformation test in allergic contact nickel dermatitis, 9

Gluckman, Eliane, Didier-Jean, Liliane, Bussel, Annette; Saurat, J.H. and: graft versus host reaction and lichen planus-like eruption in man, 591

Goncalves, J.C.A., 5-fluorouracil in the treatment of common warts of the hands, 89

Goncalves, J.C.A., treatment of solar keratoses with a 5-fluorouracil and salicylic acid varnish, 85

Goodwin, P., Hamilton, Susan, and Fry, L., cell cycle of psoriasis (letter), 230

Goolamali, S.K., Burton, J.L., Plummer, N.A., Shuster, S.; Thody, A.J. and: plasma β-MSH levels in acne vulgaris, 43

Grahame, R., Pope, F.M.; Harvey, W. and: cutaneous extensibility in pseudo-xanthoma elasticum, 679

Grasso, P.; Ingram, A.J. and: patch testing in the rabbit using a modified human patch test method. Application of histological and visual assessment, 131

Gray, Elizabeth S., Robbie, Rosalene B., Donald, D., Horne, C.H.W.; Main, R.A. and: smooth muscle antibodies and alopecia areata, 389

Grice, Katherine, Sattar, H., Casey, T. and Baker, H., an evaluation of Na$^+$, Cl$^-$ and pH ion-specific electrodes in the study of the electrolyte contents of epidermal transudate and sweat, 511

Griffiths, W.A.D.; Presbury, D.G.C. and: coeliac disease with primary cutaneous amyloidosis, 109

Grove, D.I., Reid, J.G. and Forbes, I.J., humoral and cellular immunity in atopic eczema, 611

Gunawardena, D.A., Gunawardena, K.A., Ratnayaka, R.M.R.S. and Vasanthanathan, N.S., the clinical spectrum of Sweet's syndrome (acute febrile neutrophilic dermatosis)—a report of eighteen cases, 363

Halprin, K.M., Adachi, K., Levine, V.; Yoshikawa, K. and: cyclic AMP in skin: effects of acute ischaemia, 249

Halprin, K.M., Adachi, K., Levine, V.; Yoshikawa, K. and: micro-determination of cyclic AMP levels in human epidermis, dermis and hair follicles, 241

Halprin, K.M., Adachi, K., Levine, V.; Yoshikawa, K. and: on the lack of response to catecholamine stimulation by the adenyl cyclase system in psoriatic lesions, 619

Halprin, K.M., Hsia, S.L.; Mui, M.M. and: further studies on adenyl cyclase in psoriasis, 255

Halprin, K.M., Yoshikawa, K., Levine, V.; Adachi, K. and: prostaglandins and cyclic AMP in epidermis, 381

Hamilton, Susan; Fry, L.; Goodwin, P. and: cell cycle of psoriasis (letter), 230

Handa, F., Kumar, K. and Kumar, R., a case of variegate porphyria in an Indian, 347

Hanna, N., Ilkomy, M.; Mohareb, R. and: study of a new anomaly of the vermilion border of the lip, 559

Harper, R.A.; Flaxman, B.A. and: primary cell culture for biochemical studies of human keratinocytes, 305

Harvey, W., Pope, F.M. and Grahame, R., cutaneous extensibility in pseudo-xanthoma elasticum, 679

Hell, Elizabeth; Hodgson, C. and: the effect of the activity of psoriasis on the response to autologous ground skin extract, 519

Hellier, F., infections in diabetes (letter), 480

Hidano, A. and Kobayashi, T., adnexal polyp of neonatal skin, 659

Hilal, N.S., McKellar, J.F., Phillips, G.O.; Davies, A.K. and: photochemistry of tetrachlorosalicylanilide and its relevance to the persistent light reactor, 143

Hodgson, C. and Hell, Elizabeth: the effect of the activity of psoriasis on the response to autologous ground skin extract, 519

Holgate, M.C., the age-of-onset of psoriasis and the relationship to parental psoriasis, 443

Holt, P.J.A., Marks, R. and Waddington, E., 'pseudomorphoea'—a side effect of subcutaneous corticosteroid injection, 689

Holt, Shirley, Rowell, N.R.; Hughes, P. and: the modifying effect of autologous serum on leukocyte migration inhibition by liver antigens in systemic lupus erythematosus, 401

Hood, A.J.K., Dobson, R.M., Taylor, R.N.; Carslaw, R.W. and: mites in the environment of cases of Norwegian scabies, 333

Horne, C.H.W., Robbie, Rosalene B., Gray, Elizabeth S., Donald, D.; Main, R.A. and: smooth muscle antibodies and alopecia areata, 389

Hsia, S.L., Halprin, K.M.; Mui, M.M. and: further studies on adenyl cyclase in psoriasis, 255
Hubler, W.R., Jr.; Wolf, J.E. and: tumour angiogenic factor associated with subcutaneous lymphoma, 273
Hughes, P., Holt, Shirley, and Rowell, N.R., the modifying effect of autologous serum on leukocyte migration inhibition by liver antigens in systemic lupus erythematosus, 401
Hunter, J.A.A., Moore, M.R.; Allen, B.R. and: lead and the skin, 715
Huntsman, R.G., Ellis, L., Said, M.I.; Afifi, A.M. and: high dose ascorbic acid in the management of thalassaemia leg ulcers—a pilot study, 339
Hursthouse, M.W., a controlled trial on the use of topical 5-fluorouracil on viral warts, 93

Ingram, A.J. and Grasso, P., patch testing in the rabbit using a modified human patch test method. Application of histological and visual assessment 131
Inouye, Joyce C., McGerity, J.L., Lewis, C.W.; Jones, H.E. and: atopic disease and serum immunoglobulin-E, 17

Jablonska, Stefania: measurement of sensory chronaxie as a diagnostic procedure in scleroderma, 223
Jarrett, A.; Spearman, R.I.C. and: bio-assay of corticosteroids for topical application, 581
Johnson, G.D., Kumar, Parveen, Clark, M.L., Marks, R.; Lancaster-Smith, M. and: antireticulin antibodies in dermatitis herpetiformis and adult coeliac disease, 37
Jones, H.E., Inouye, Joyce C., McGerity, J.L. and Lewis, C.W., atopic disease and serum immunoglobulin-E, 17
Jordan, J.M., Whitlock, F.A.; Robertson, I.M. and: Emotions and skin (II)—the conditioning of scratch responses in cases of lichen simplex, 407
Jordon, R.E., Schroeter, A.L. and Winkelmann, R.K., dermal-epidermal deposition of complement components and properdin in systemic lupus erythematosus, 263

Kaidbey, K.H., Kligman, A.M. and Yoshida, H., effects of intensive application of retinoic acid on human skin, 693
Kalsbeek, G.L.; Van Lis, J.M.J. and: the interaction of Concanavalin A and the surface coat of stratified squamous epithelium, 27
Kamalam, A., Yesudian, P., Augustine, S.M., Premalatha, S.; Thambiah, A.S. and: tylosis following post-corrosive stricture of the oesophagus, 219
Kana, M.W., mucocutaneous leishmaniasis in guinea-pigs inoculated intravenously with *L. enriettii*, 663
Kanan, M.W. and Ryan, T.J., endonasal localization of blood-borne viable and non-viable particulate matter, 475
Katz, S.A.; Samitz, M.H. and: nickel dermatitis hazards from prostheses. *In vivo* and *in vitro* solubilization studies, 287
Kligman, A.M.; Robinson, T.A. and: treatment of solar keratoses of the extremities with retinoic acid and 5-fluorouracil, 703
Kligman, A.M., Yoshida, H.; Kaidbey, K.H. and: effects of intensive application of retinoic acid on human skin, 693
Knudsen, E.A., the areal extent of dermatophyte infection, 413
Kobayashi, T.; Hidano, A. and: adnexal polyp of neonatal skin, 659
Konrad, K., Wolff, K.; Schnait, F.G. and: erythropoietic protoporphyria — submicroscopic events during the acute photosensitivity flare, 545
Kumar, K., Kumar, R.; Handa, F. and: a case of variegate porphyria in an Indian, 347
Kumar, Parveen, Clark, M.L., Marks, R., Johnson, G.D.; Lancaster-Smith, M. and: antireticulin antibodies in dermatitis herpetiformis and adult coeliac disease, 37

Lachmann, P.J., complement deficiencies, 593
Lancaster-Smith, M., Kumar, Parveen, Clark, M.L., Marks, R. and Johnson, G.D., antireticulin antibodies in dermatitis herpetiformis and adult coeliac disease, 37
Leigh, I.M. and Ramsay, C.A., localized heat urticaria treated by inducing tolerance to heat, 191
Lemholt, K.; Nyfors, A. and: psoriasis in children, 437
Leppard, Barbara, and Sneddon, I.B., milia occurring in lichen sclerosus et atrophicus, 711
Levine, V., Adachi, K., Halprin, K.M.; Yoshikawa, K. and: cyclic AMP in skin: effects of acute ischaemia, 249
Levine, V., Adachi, K., Halprin, K.M.; Yoshikawa, K. and: micro-determination of cyclic AMP levels in human epidermis, dermis and hair follicles, 241
Levine, V., Adachi, K., Halprin, K.M.; Yoshikawa, K. and: on the lack of response to catecholamine stimulation by the adenyl cyclase system in psoriatic lesions, 619
Levine, V., Yoshikawa, K., Halprin, K.M.; Adachi, K. and: prostaglandins and cyclic AMP in epidermis, 381
Lewis, C.W., Inouye, Joyce C., McGerity, J.L.; Jones, H.E. and: atopic disease and serum immunoglobulin-E, 17
Liddell, K. and White, J., the smell of cancer, 215
Liddell, K., White, J. and Caldwell, I.W., seborrhoeic keratoses and carcinoma of the large bowel, 449
Long, V.J.W., phospholipase A and lysophospholipase activity of the epidermis, 603

Macadam, R.F., Vetters, J.M. and Saikia, N.K., a search for microtubular inclusions in endothelial cells in a variety of skin diseases, 175
McGerity, J.L., Inouye, Joyce, C., Lewis, C.W.; Jones, H.E. and: atopic disease and serum immunoglobulin-E, 17
McKellar, J.F., Hilal, N.S., Phillips, G.O.; Davies, A.K. and: photochemistry of tetrachlorosalicylanilide and its relevance to the persistent light reactor, 143

McKenzie, A.W.; Whitefield, M. and: a new formulation of 0·1% hydrocortisone cream with vasoconstrictor activity and clinical effectiveness, 585

Mackenzie, I.C. and Squier, C.A., cytochemical identification of ATPase-positive Langerhans cells in EDTA-separated sheets of mouse epidermis, 523

MacKie, Rona M., Finn, O.A., McQueen, A., MacSween, R.N.M.; Woolfson, H. and: serum anti-tumour antibodies and auto-antibodies in vitiligo, 395

MacLeod, T.M. and Frain-Bell, W., a study of chemical light screening agents, 417

MacLeod, T.M. and Frain-Bell, W.: a study of physical light screening agents, 149

McQueen, A., Finn, O.A., MacKie, Rona M., MacSween, R.N.M.; Woolfson, H. and: serum anti-tumour antibodies and auto-antibodies in vitiligo, 395

MacSween, R.N.M., Finn, O.A., MacKie, Rona M. McQueen, A.; Woolfson, H. and: serum antitumour antibodies and auto-antibodies in vitiligo, 395

Main, R.A., Robbie, Rosalene B., Gray, Elizabeth S., Donald, D. and Horne, C.H.W., smooth muscle antibodies and alopecia areata, 389

Malina, L. and Chlumsky, J., oestrogen-induced familial porphyria cutanea tarda, 707

Mann, S.J.; Blank, F. and: *Trichophyton rubrum* infections according to age, anatomical distribution and sex, 171

Mannoni, P., Revuz, J., Dreyfus, B., Rochant, H.; Touraine, R. and: graft-versus-host reaction and lichen planus, 589

Marks, R., Abell, E. and Nishikawa, T., the junctional zone beneath migrating epidermis, 311

Marks, R., Kumar, Parveen, Clark, M.L., Johnson, G.D.; Lancaster-Smith, M. and: antireticulin antibodies in dermatitis herpetiformis and adult coeliac disease, 37

Marks, R. and Pearse, A.D., surfometry. A method of evaluating the internal structure of the stratum corneum, 651

Marks, R.; Tree, S. and: an explanation for the 'placebo' effect of bland ointment bases, 195

Marks, R.; Waddington, E; Holt, P.J.A. and: 'pseudomorphoea'—a side effect of subcutaneous corticosteroid injection, 689

Masawe, A.J.E., Mhalu, F.S.; Nsanzumuhire, H. and: bacteriological eco-system of the skin of children in an African tropical environment (Tanzania), 77

Meyrick, Gay, Pye, R.J., Shuster, S.; Burton, J.L. and: the sebum excretion rate in rosacea, 541

Mhalu, F.S., Masawe, A.J.E.; Nsanzumuhire, H. and: bacteriological eco-system of the skin of children in an African tropical environment (Tanzania), 77

Mier, P.D., earliest description of the atopic syndrome? (letter), 359

Mier, P.D., Van Den Hurk, J. and Van Rossum, E., plasma vitamin A levels in the dyskeratoses, 73

Mobacken, H. and Romanus, M., microvascular response to local application of dequalinium chloride, 63

Mohareb, R., Elkomy, M. and Hanna, N., study of a new anomaly of the vermilion border of the lip, 559

Moore, M.R., Hunter, J.A.A.; Allen, B.R. and: lead and the skin, 715

Morison, W.L., anti-viral treatment of warts, 97

Morison, W.L., viral warts, herpes simplex and herpes zoster in patients with secondary immune deficiencies and neoplasms, 625

Moschella, S.L.; Crutcher, W.A. and: double-blind controlled crossover high-dose study of Azaribine in psoriasis, 199

Mui, M.M., Hsia, S.L. and Halprin, K.M., further studies on adenyl cyclase in psoriasis, 255

Neville, E.A. and Finn, O.A., psoriasiform napkin dermatitis—a follow-up study, 279

Nishikawa, T., Abell, E.; Marks, R. and: the junctional zone beneath migrating epidermis, 311

Nsanzumuhire, H., Masawe, A.J.E. and Mhalu, F.S., bacteriological eco-system of the skin of children in an African tropical environment (Tanzania), 77

Nyfors, A. and Lemholt, K., psoriasis in children, 437

Okoro, A.N., albinism in Nigeria, 485

O'Shea, J.A.; Bettley, F.R. and: the absorption of arsenic and its relation to carcinoma, 563

Park, J.R., De Bats, A. and Rhodes, E.L., the effect of transfer factor on the *in vitro* migration of tuberculin stimulated white cells from sarcoid and normal subjects, 535

Pearse, A.D.; Marks, R. and: surfometry. A method of evaluating the internal structure of the stratum corneum, 651

Petkov, I.; Andreev, V.C. and: skin manifestations associated with tumours of the brain, 675

Phillips, G.O., Hilal, N.S., McKellar, J.F.; Davies, A.K. and: photochemistry of tetrachlorosalicylanilide and its relevance to the persistent light reactor, 143

Plummer, N.A., Goolamali, S.K., Burton, J.L., Shuster, S.; Thody, A.J. and: plasma β-MSH levels in acne vulgaris, 43

Pope, F.M., historical evidence for the genetic heterogeneity of pseudoxanthoma elasticum, 493

Pope, F.M., Grahame, R.; Harvey, W. and: cutaneous extensibility in pseudo-xanthoma elasticum, 679

Premalatha, S., Yesudian, P., Augustine, S.M., Kamalam, A.; Thambiah, A.S. and: tylosis following post-corrosive stricture of the oesophagus, 219

Presbury, D.G.C. and Griffiths, W.A.D., coeliac disease with primary cutaneous amyloidosis, 109

Pye, R.J., Meyrick, Gay, Shuster, S.; Burton, J.L. and: the sebum excretion rate in rosacea, 541

Ramnarain, D.; Abell, E. and: epidermal antigens in lichen planus, 631

Ramsay, C.A. and Giannelli, F., the erythemal action spectrum and DNA repair synthesis in xeroderma pigmentosum, 49

Ramsay, C.A.; Leigh, I.M. and: localized heat urticaria treated by inducing tolerance to heat, 191

Randall, Valerie, Ebling, Erika, Skinner, J.; Ebling, F.J. and: the sebotrophic action of growth hormone (BGH) in the rat, 325

Ratnayaka, R.M.R.S., Gunawardena, K.A., Vasanthanathan, N.S.; Gunawardena, D.A. and: the clinical spectrum of Sweet's syndrome (acute febrile neutrophilic dermatosis)—a report of eighteen cases, 363

Reid, J.G., Forbes, I.J.; Grove, D.I. and: humoral and cellular immunity in atopic eczema, 611

Revuz, J., Dreyfus, B., Rochant, H., Mannoni, P.; Touraine, R. and: graft-versus-host reaction and lichen planus, 589

Rhodes, E.L., De Bats, A.; Park, J.R. and: the effect of transfer factor on the *in vitro* migration of tuberculin stimulated white cells from sarcoid and normal subjects, 535

Riley, P.A., growth inhibition in normal mammalian melanocytes *in vitro*, 291

Robbie, Rosalene, B., Gray, Elizabeth S., Donald, D., Horne, C.H.W.; Main, R. and: smooth muscle antibodies and alopecia areata, 389

Robertson, I.M., Jordan, J.M. and Whitlock, F.A., emotions and skin (II)—the conditioning of scratch responses in cases of lichen simplex, 407

Robinson, T.A. and Kligman, A.M., treatment of solar keratoses of the extremities with retinoic acid and 5-fluorouracil, 703

Rochant, H., Revuz, J., Dreyfus, B., Mannoni, P.; Touraine, R. and: graft-versus-host reaction and lichen planus, 589

Romanus, M.; Mobacken, H. and: microvascular response to local application of dequalinium chloride, 63

Rook, A., Hair. (review) I. The clinical significance of abnormal hair-slope patterns—Trichoglyphics, 239. II. Racial and other genetic variations in hair form, 599

Rottier, P.B., Baart De La Faille-Kuyper, E.H.; Baart De La Faille, H. and: solar urticaria, 101

Rowell, N.R., Holt, Shirley; Hughes, P. and: the modifying effect of autologous serum of leukocyte migration inhibition by liver antigens in systemic lupus erythematosus, 401

Rudolph, R.I., piezogenic pedal papules (letter), 113

Ryan, T.J.; Kanan, M.W. and: endonasal localization of blood-borne viable and non-viable particulate matter, 475

Said, M.I., Ellis, L., Huntsman, R.G.; Afifi, A.M. and: high dose ascorbic acid in the management of thalassaemia leg ulcers—a pilot study, 339

Saikia, N.K., Vetters, J.M.; Macadam, R.F. and: a search for microtubular inclusions in endothelial cells in a variety of skin diseases, 175

Samitz, M.H. and Katz, S.A., nickel dermatitis hazards from prostheses. *In vivo* and *in vitro* solubilization studies, 287

Sarkany, I.; Gaylarde, P.M. and: cell migration and DNA synthesis in organ culture of human skin, 375

Sattar, H., Casey, T., Baker, H.; Grice, Katherine, and: an evaluation of Na^+, Cl^- and pH ion-specific electrodes in the study of the electrolyte contents of epidermal transudate and sweat, 511

Saurat, J.H., Didier-Jean, Liliane, Gluckman, E. and Bussel, Annette: graft-versus-host reaction and lichen planus-like eruption in man, 591

Schnait, F.G., Wolff, K. and Konrad, K., erythropoietic protoporphyria—submicroscopic events during the acute photosensitivity flare, 545

Schroeter, A.L., Winkelmann, R.K.; Jordon, R.E. and: dermal-epidermal deposition of complement components and properdin in systemic lupus erythematosus, 263

Seah, P.P. and Fry, L., immunoglobulins in the skin in dermatitis herpetiformis and their relevance in diagnosis, 157

Shuster, S., Goolamali, S.K., Burton, J.L., Plummer, N.A.; Thody, A.J. and: plasma β-MSH levels in acne vulgaris, 43

Shuster, S., Pye, R.J., Meyrick, Gay; Burton, J.L. and: the sebum excretion rate in rosacea, 541

Singh, G., traction alopecia in Sikh boys (letter), 232

Skinner, J.; Ebling, F.J. and: the removal and restitution of hair fat in the rat, 321

Skinner, J., Ebling, Erika, Randall, Valerie; Ebling, F.J. and: the sebotrophic action of growth hormone (BGH) in the rat, 325

Sneddon, I.B.; Leppard, Barbara, and: milia occurring in lichen sclerosus et atrophicus, 711

Spearman, R.I.C. and Jarrett, A., bio-assay of corticosteroids for topical application, 581

Squier, C.A.; Mackenzie, I.C. and: cytochemical identification of ATPase-positive Langerhans cells in EDTA-separated sheets of mouse epidermis, 523

Stankler, L. and Dave, Vinay K., the influence of treatment of the paralesional skin with dithranol on healing in psoriasis, 57

Tan, R.S.H. and Delaney, T.J., circular regrowth in alopecia areata (letter), 233

Taylor, R.N., Dobson, R.M., Hood, A.J.K.; Carslaw, R.W. and: mites in the environment of cases of Norwegian scabies, 333

Thambiah, A.S., Yesudian, P., Augustine, S.M., Kamalam, A. and Premalatha, S., tylosis following post-corrosive stricture of the oesophagus, 219

Thiele, F.A.J., chemical aspects of hair loss and its cosmetological significance, 355

Thiele, F.A.J., measurements of the surface of the skin (thesis summary), 115

Thody, A.J., Goolamali, S.K., Burton, J.L., Plummer, N.A. and Shuster, S., plasma β-MSH levels in acne vulgaris, 43

Thorne, G., mycetoma and *Trichophyton rubrum* (letter), 231

Touraine, R., Revuz, J., Dreyfus, B., Rochant, H.

and Mannoni, P., graft-versus-host reaction and lichen planus, 589

Tree, S. and Marks, R., an explanation for the 'placebo' effect of bland ointment bases, 195

Usher, N., Dave, V.K., Anderson, Jeannette; Woodrow, J.C. and: the HL-A system and psoriasis, 427

Van Den Hurk, J., Van Rossum, E.; Mier, P.D. and: plasma vitamin A levels in the dyskeratoses, 73

Van Lis, J.M.J. and Kalsbeek, G.L., the interaction of Concanavalin A and the surface coat of stratified squamous epithelium, 27

Van Rossum, E., Van Den Hurk, J.; Mier, P.D. and: plasma vitamin A levels in the dyskeratoses, 73

Vasanthanathan, N.S., Gunawardena, K.A., Ratnayaka, R.M.R.S.; Gunawardena, D.A. and: the clinical spectrum of Sweet's syndrome (acute febrile neutrophilic dermatosis)—a report of eighteen cases, 363

Verbov, J., anonychia with bizarre flexural pigmentation—an autosomal dominant dermatosis, 469

Verhagen, A.R. and Woerdeman, M.J., post-inflammatory elastolysis and cutis laxa, 183

Vetters, J.M., Saikia, N.K.; Macadam, R.F. and: a search for microtubular inclusions in endothelial cells in a variety of skin diseases, 175

Waddington, E., Marks, R.; Holt, P.J.A. and: 'pseudomorphoea'—a side effect of subcutaneous corticosteroid injection, 689

Ward, Joanna, M., *M. fortuitum* and *M. chelonei*—fast growing mycobacteria, 453

Watson, B.W., Canti, G.; Woodrough, R.E. and: electrical potential difference between basal cell carcinoma, benign inflammatory lesions and normal tissue, 1

Weinstein, G.D., on the cell cycle of psoriasis (letter), 229

White, J.E., Caldwell, I.W.; Liddell, K. and: seborrhoeic keratoses and carcinoma of the large bowel, 449

White, J.E.; Liddell, K. and: the smell of cancer, 215

Whitefield, M. and McKenzie, A.W., a new formulation of 0·1% hydrocortisone cream with vasoconstrictor activity and clinical effectiveness, 585

Whitlock, F.A., Jordan, J.M.; Robertson, I.M. and: emotions and skin (II)—the conditioning of scratch responses in cases of lichen simplex, 407

Wilson, H.T.H., a fixed drug eruption due to paracetamol, 213

Winkelmann, R.K., Schroeter, A.L.; Jordon, R.E. and: dermal-epidermal deposition of complement components and properdin in systemic lupus erythematosus, 263

Wishart, J.M., Wegener's granulomatosis—controlled by azathioprine and corticosteroids, 461

Wissler, F. and Carruthers, C., heterogeneity of bovine snout epidermal prekeratin, 647

Woerdeman, M.J.; Verhagen, A.R. and: post-inflammatory elastolysis and cutis laxa, 183

Wolf, J.E. and Hubler, W.R., Jr., tumour angiogenic factor associated with subcutaneous lymphoma, 273

Wolff, K., Konrad, K.; Schnait, F.G. and: erythropoietic protoporphyria—submicroscopic events during the acute photosensitivity flare, 545

Woodrough, R.E., Canti, G. and Watson, B.W., electrical potential difference between basal cell carcinoma, benign inflammatory lesions and normal tissue, 1

Woodrow, J.C., Dave, V.K., Usher, N. and Anderson, Jeanette: the HL-A system and psoriasis, 427

Woolfson, H., Finn, O.A., MacKie, Rona M., McQueen, A. and MacSween, R.N.M., serum anti-tumour antibodies and auto-antibodies in vitiligo, 395

Wrench, Rosanne, and Britten, A.Z., evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test. I. High and low temperature tars and their constituents, 569. II. Tar oil acids, 575

Yesudian, P., Augustine, S.M., Kamalam, A., Premalatha, S.; Thambiah, A.S. and: tylosis following post-corrosive stricture of the oesophagus, 219

Yoshida, H., Kligman, A.M.; Kaidbey, K.H. and: effects of intensive application of retinoic acid on human skin, 693

Yoshikawa, K., Adachi, K., Halprin, K.M. and Levine, V., cyclic AMP in skin: effects of acute ischaemia, 249

Yoshikawa, K., Adachi, K., Halprin, K.M. and Levine, V., micro-determination of cyclic AMP levels in human epidermis, dermis and hair follicles, 241

Yoshikawa, K., Adachi, K., Halprin, K.M. and Levine, V., on the lack of response to catecholamine stimulation by the adenyl cyclase system in psoriatic lesions, 619

Yoshikawa, K., Halprin, K.M., Levine, V.; Adachi, K. and: prostaglandins and cyclic AMP in epidermis, 381

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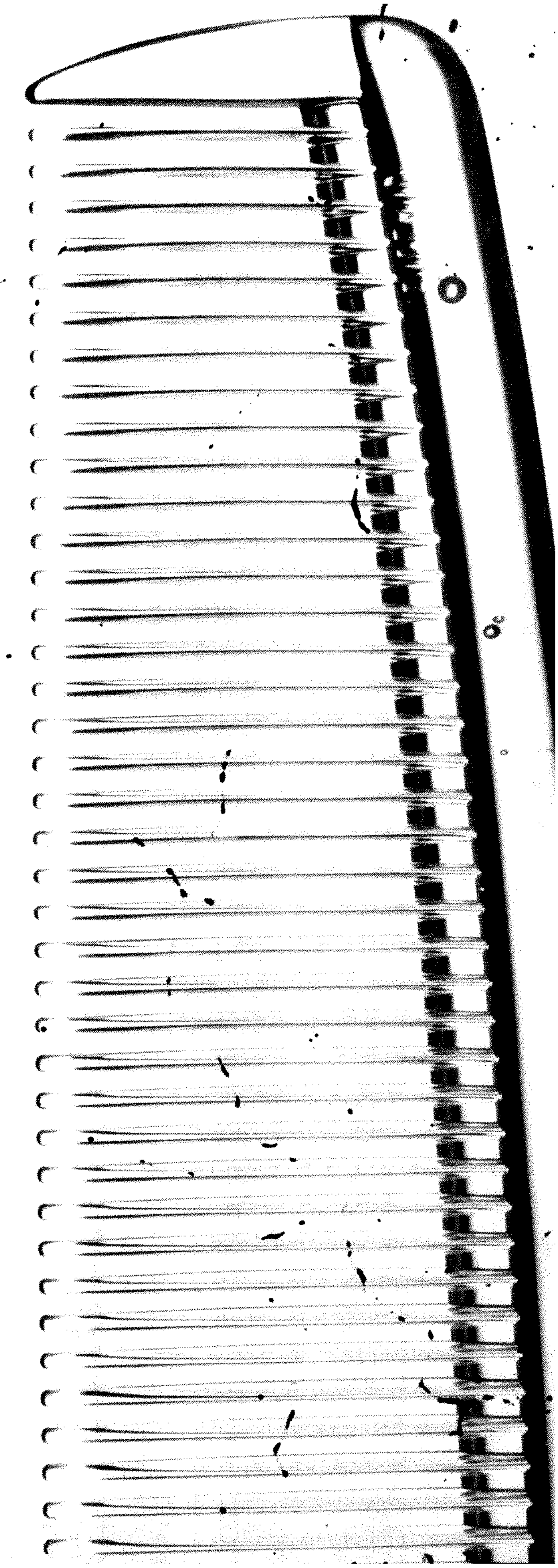

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Outline of Contents

Dermatoses due to fungi
Dermatoses due to viruses
Treponematoses
Dermatoses due to bacteria
Dermatoses due to animals
Dermatoses due to malnutrition
Exogenous and endogenous eczemas, lichen simplex and prurigo
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Index Vol. 149, No. 5, 1974

Barrière, H.; Litoux, P. et Géraut, C. (Nantes): Etude des cellules cornées du psoriasis en microscopie électronique à balayage
(Study of Psoriasic Horny Cells with the Scanning Electron Microscope)

Pierard, G.E.; Hermanns, J.F. et Lapière, C.M. (Liège): Stéréologie de l'interface dermo-épidermique. Observation de la plasticité de la membrane basale au microscope électronique à balayage
(Stereology of the Dermo-Epidermal Junction)

Förster, F.J.; Kallenberg, L.; Fertig, U., and Leonhardi, G. (Frankfurt a.M.): Subcellular Distribution and Cytoplasmatic Kinetic Properties of Acid Phosphatase of Normal Stratum Corneum and Psoriatic Scales

Mier, P.D. and Hurk, J.J.M.A. van den (Nijmegen): Direct Biochemical Assay of the Lipase Activity of Epidermis

Hellgren, L. and Larsson, K. (Gothenburg): On the Effect of Urea on Human Epidermis

Young, E. (Utrecht): Contact Hypersensitivity to Metallic Gold

The Interesting Case · Der interessante Fall · Un cas intéressant

Vermeer, B.J. and Heremans, G.F.P. (Leiden): A Case of Growth Retardation and Cushing's Syndrome Due to Excessive Application of Betamethasone-17-Valerate Ointment

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm³	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
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British Journal of Dermatology, Volume 92, Number 6, June 1975

CONTENTS

Clinical and Laboratory Investigations

Page

Phospholipase A and Lysophospholipase Activity of the Epidermis 603
V. J. W. LONG

Humoral and Cellular Immunity in Atopic Eczema 611
D. I. GROVE, J. G. REID AND P. J. FORBES

On the Lack of Response to Catecholamine Stimulation by the Adenyl Cyclase System in Psoriatic Lesions 619
K. YOSHIKAWA, K. ADACHI, K. M. HALPRIN AND V. LEVINE

Viral Warts, Herpes Simplex and Herpes Zoster in Patients with Secondary Immune Deficiences and Neoplasms 625
WARWICK L. MORISON

Epidermal Antigens in Lichen Planus 631
E. ABELL AND D. RAMNARAIN

Light and Electron Microscopic Differentiation of Amyloid and Colloid or Hyaline Bodies 637
H. EBNER AND W. GEBHART

Heterogeneity of Bovine Snout Epidermal Prekeratin 647
F. WISSLER AND C. CARRUTHERS

Surfometry—a Method of Evaluating the Internal Structure of the Stratum Corneum 651
R. MARKS AND A. D. PEARSE

Adnexal Polyp of Neonatal Skin 659
A. HIDANO AND T. KOBAYASHI

Mucocutaneous Leishmaniasis in Guinea-pigs Inoculated Intravenously with *Leishmania enriettii* 663
M. W. KANAN

Skin Manifestations Associated with Tumours of the Brain 675
V. C. ANDREEV AND I. PETKOV

Cutaneous Extensibility in Pseudo-xanthoma Elasticum 679
W. HARVEY, F. M. POPE AND R. GRAHAME

Chronic Paronychia and Psoriasis 685
SALOMON GANOR

'Pseudomorphoea'—a Side Effect of Subcutaneous Corticosteroid Injection 689
P. J. A. HOLT, R. MARKS AND [illegible] WADDINGTON

Pharmacology and Treatment

Effects of Intensive Application of Retinoic Acid on Human Skin 693
KAYS H. KAIDBEY, ALBERT M. KLIGMAN AND HIKOTARO YOSHIDA

Treatment of Solar Keratoses of the Extremities with Retinoic Acid and 5-fluorouracil 703
TERRANCE A. ROBINSON AND ALBERT M. KLIGMAN

Case Reports

Oestrogen-induced Familial Porphyria Cutanea Tarda 707
L. MALINA AND J. CHLUMSKY

Milia Occurring in Lichen Sclerosus et Atrophicus 711
BARBARA LEPPARD AND I. B. SNEDDON

Comment

Lead and the Skin 715
B. R. ALLEN, M. R. MOORE AND J. A. A. HUNTER

Book Reviews 720

News and Notices 7[illegible]

Index to Volume 92 723